Supplement to:

CXCL12-abundant Reticular Cells are the Major Source of IL-6 Upon LPS-stimulation and Thereby Regulate Hematopoiesis

Rahel C. Gerosa,1* Steffen Boettcher,1* Larisa V. Kovtonyuk,1 Annika Hausmann,2 Wolf-Dietrich Hardt,2 Juan Hidalgo,3,4 César Nombela-Arrieta,1 and Markus G. Manz1

1Department of Medical Oncology and Hematology, University of Zurich and University Hospital Zurich, Zurich, Switzerland
2The Institute of Microbiology, Department of Biology, ETH Zurich, Switzerland
3Animal Physiology Unit, Department of Cellular Biology, Physiology and Immunology, Faculty of Biosciences, Universitat Autònoma de Barcelona, Spain
4Institute of Neurosciences, Universitat Autònoma de Barcelona, Spain

Short Title: BM CAR cells regulate hematopoiesis via IL-6
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Corresponding Author:
Markus G. Manz, MD
Department of Medical Oncology and Hematology
University of Zurich and University Hospital Zurich
Raemistrasse 100
CH-8091 Zurich
Switzerland
Phone: +41-44-255 3899
Fax: +41-44-255 4560
markus.manz@usz.ch

R.C.G. and S.B. contributed equally to this study*.
# Supplementary methods

## Primer Sequences

| Gene | Forward | Reverse |
|------|---------|---------|
| Cxcl12 | GCATCAGTGACGGTAAACCA | GTTTAAAGCTTCTCCAGGTACTC |
| Fli3l | AGTTCTGCTTCTAGTCAGG | TTGATATGCTGTACCCTCCTC |
| Csf3 | GCCTTCTGCTTAAATGTCCTG | CTGAGTACCTGTGTCTGCTG |
| Csf2 | CACTGTCAGGTGAATGAGAG | TTTAGATATGCTGTACCCTCCTC |
| Il1a | CAAGGGGAGATTCTGAGAAGAG | GGGTTGATGGTCTCTTCCAG |
| Il1b | CCTTTGAGGAGAGCAGGAGCCATCC | GCTGCATCAGACAGCCTTGCAGGTC |
| Il3 | AGCTCCAGAACCTGAACTC | ACTTCTCTGGCCTTTCCCAC |
| Il6 | CAGTCAGAGAGACTTCCATCC | CTGACTGTGACAGGTCTTGGT |
| Il7 | GACACTTTGGATTCCCCGAGGAGAAC | GACTGACTGTGACAGGTCTTGGT |
| Il8 | AACTCAGCTACAGGGCGGACTTCA | TCCCAGTCAATCTTCTTTGGTT |
| Kitl | CAGAAAATAGTCTTCTTAATCCCTG | ACAGTAAATAGTGAGCTG |
| Tlr1 | CATCACGACAGGAGGAG | GCTGCATCAGACAGCCTTGCAGGTC |
| Tlr2 | TCAGACAGAGGACAGAAGACCTCAG | GCATCAGACAGGAGGAG |
| Tlr3 | GATAAGGGAGAGAGCTTTTACAGG | CAGATAGAGAGAGGAGTGC |
| Tlr5 | CCAAGTCTGGATCATGCCACTC | ATGGTGATGGGATTGGTTGGT |
| Tlr6 | AAGGAAATGTCTTGGATAGTAAGAGG | ATCCAGTGAATCTTCTTTGGCT |
| Tlr7 | TTGAAGGAGAGAGGACTGTTGGCTC | AAATTTGCTTCTTCCGTGCA |
| Tlr8 | CTCTTCCCTTGTCTTATGAACATG | ATGTGTAATGGCATTGTCTG |
| Tlr9 | CAGTACGAGGATCTTCTGTC | TCAGGTAGAGGATCTTCTG |
| Tnf | CACTGTTAAGACCAAGCTTACAGG | TGGGACTGTGGGGTAGTAGA |
| Thpo | GAACCGGCTTCTCAGAGG | TGGGACTGTGGGGTAGTAGA |

### Generation of reciprocal chimeras

WT (CD45.1+ and CD45.2+), Il6−/− and Tlr4−/− (CD45.1+ and CD45.2+) mice were used to generate reciprocal chimeras. Mice were lethally irradiated with 950 cGy and transplanted intravenously with 5 x 10^6 total BM cells. All animals were maintained at the University Hospital Zurich animal facility and treated in accordance with guidelines of the Swiss Federal Veterinary Office. Experiments and procedures were approved by the Veterinäramt des Kantons Zurich, Switzerland.
**Isolation of BM non-hematopoietic cells**

Femurs, tibias and hip bones were removed, BM was flushed and cells were resuspended with digestion medium (DMEM/10% FCS/10mM HEPES/0.4% collagenase II (Worthington)/0.02% DNase I (Worthington)). Bones were cut into small pieces and combined with flushed BM in a total of 5 ml digestion medium. Bones and BM were incubated for 45 min at 37°C on a shaker and afterwards the cell suspension was filtered through a 70um cell strainer. LS MACS columns (Miltenyi Biotec) were then used to enrich the cell suspension for CD45\(^{-}\)Ter119\(^{-}\) cells following the supplier’s protocols. The resulting cell population was then sorted on a FACS Aria II.

**Competitive transplantation assay**

Whole bone marrow (WBM) were isolated from PBS- and LPS-treated (according to Figure 6A) Lepr\(^{Cre}\);Il6\(^{fl/fl}\) and control mice (CD45.2). Recipient wildtype mice (CD45.1) were lethally irradiated with 950 cGy and transplanted intravenously with 4 × 10\(^5\) WBM cells from above donor mice alongside with equal numbers of competitor cells (CD45.1/2). Peripheral blood chimerism was measured based on CD45.2 expression 4, 8, 12, and 16 weeks after transplantation. All animals were maintained at the University Hospital Zurich animal facility and treated in accordance with guidelines of the Swiss Federal Veterinary Office. Experiments and procedures were approved by the Veterinäramt des Kantons Zurich, Switzerland.
Supplemental figure legends

Supplemental Figure 1. Expression of hematopoietic growth factors and inflammatory cytokines in non-hematopoietic BM stromal cell subpopulations following a single LPS injection.

(A) Gene expression of 12 hematopoietic growth factors and inflammatory cytokines from PBS-, LPS- or poly(I:C)-injected WT mice. Relative expression normalized to Gapdh from RT-qPCR is depicted. Kitl, kit ligand; Flt3l, FMS-like tyrosine kinase 3 ligand; Thpo, thrombopoietin; Csf2, colony stimulating factor 2 (granulocyte-macrophage); Csf3, colony stimulating factor 3 (granulocyte); Tnf, tumor necrosis factor; Il1a, interleukin 1 alpha; Il1b, interleukin 1 beta; Il3, interleukin 3; Il6, interleukin 6; Ifna, interferon alpha; Ifnb, interferon beta.

Supplemental Figure 2. Hematopoietic response in BM of Il6−/− mice following short-term LPS treatment.

(A) Graphical scheme depicting experimental outline for the induction of acute inflammation. (B) BM cellularity and absolute numbers per hind leg of immunophenotypically-defined LT-HSCs, HPCs, GMPs, CD11b*Gr1high and CD11b*Gr1low cells in LPS (red bars) or PBS (white bars) treated WT and Il6−/− mice according to the treatment schedule depicted under Figure S3A. Data from three independent experiments are shown. (ns, non-significant; *, p=0.05).

Supplemental Figure 3. Hematopoietic response in BM of Il6−/− and LeprCre;Il6fl/fl mice upon chronic-repetitive LPS treatment.

(A) Graphical scheme depicting experimental outline for modeling chronic inflammation by repetitive LPS injections over a period of 3 weeks followed by analysis of different hematopoietic cell types by FACS. This schedule is applicable to experimental data depicted in panels B and C. (B) Percentages of immunophenotypically-defined LT-HSCs, HPCs, GMPs, CD11b*Gr1high and CD11b*Gr1low cells in the BM of LPS (red bars) or PBS (white bars) treated WT and Il6−/− mice according to the treatment schedule depicted under Figure S4A. (C) Percentages of immunophenotypically-defined LT-HSCs, HPCs, GMPs, CD11b*Gr1high and CD11b*Gr1low cells in the BM of LPS (blue bars) or PBS (white bars) treated control and LeprCre;Il6fl/fl mice according to the treatment schedule depicted under Figure S4A. Data from three independent experiments are shown. (ns, non-significant; *, p=0.05; **, p<0.01; ***, p<0.001; ****, p<0.0001).

Supplemental Figure 4. Toll-like receptor 4 gene expression by non-hematopoietic stromal cell populations.
(A) Tlr4 expression as assessed by RT-qPCR in CAR cells (red bar), DCs (pooled cDCs and pDCs, grey bars), AECs, SECs, and MSCs (all black bars) isolated from steady-state WT mice. Expression values were normalized to housekeeping gene Gapdh.
Figure S1

A

- **Kitl**
  - PBS: 100%
  - LPS: 80%
  - Poly(I:C): 60%

- **Pltf**
  - PBS: 150%
  - LPS: 120%
  - Poly(I:C): 90%

- **Thpo**
  - PBS: 2.5
  - LPS: 2.0
  - Poly(I:C): 1.5

- **Csf2**
  - PBS: 20
  - LPS: 15
  - Poly(I:C): 10

- **Csf3**
  - PBS: 250
  - LPS: 200
  - Poly(I:C): 150

- **Tnf**
  - PBS: 5
  - LPS: 4
  - Poly(I:C): 3

- **Il1a**
  - PBS: 8
  - LPS: 6
  - Poly(I:C): 4

- **Il1b**
  - PBS: 15
  - LPS: 10
  - Poly(I:C): 5

- **Il3**
  - PBS: 0.8
  - LPS: 0.6
  - Poly(I:C): 0.4

- **Il6**
  - PBS: 500
  - LPS: 400
  - Poly(I:C): 300

- **Ilha**
  - PBS: 2
  - LPS: 1.5
  - Poly(I:C): 1

- **Ilnb**
  - PBS: 10
  - LPS: 8
  - Poly(I:C): 6
Figure S2

A

35 μg LPS i.p. or PBS control i.p. → BM → FACS

0 48 72 hrs

B

BM cellularity

WT     Il6-/-

PBS  LPS

CD11b^Gr1^low myeloid cells

CD11b^Gr1^high myeloid cells

IL-6

WT     Il6-/-

1x10^6  2x10^7  4x10^7  6x10^7  8x10^7  10^8

15'000  10'000  5'000

PBS  LPS

1x10^5  2x10^5  4x10^5  6x10^5  8x10^5  1x10^6

4x10^5  3x10^5  2.5x10^5  2x10^5  1.5x10^5  1x10^5

2.5x10^7  0.5x10^6  2.0x10^7
Figure S3

A

35 μg LPS i.p. or PBS control i.p.

0 2 4 7 9 11 14 16 18 19d

BM → FACS

B

LT-HSC

HPC

GMP

CD11b+Gr1<sub>low</sub>

myeloid cells

CD11b+Gr1<sub>high</sub>

myeloid cells

% of live BM cells

0.04 0.03 0.02 0.01

0.5 0.4 0.3 0.2 0.1

0.1

0.01

PBS

LPS

C

LT-HSC

HPC

GMP

CD11b<sup>+</sup>Gr1<sub>low</sub>

myeloid cells

CD11b<sup>+</sup>Gr1<sub>high</sub>

myeloid cells

% of live BM cells

0.04 0.03 0.02 0.01

0.5 0.4 0.3 0.2 0.1

0.1

0.01

PBS

LPS
Figure S4

A

Relative expression to Gapdh (%)