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Supplementary Materials for

A leukotriene-dependent spleen-liver axis drives TNF production in systemic inflammation

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at stke.sciencemag.org/cgi/content/full/14/679/eabb0969/DC1)

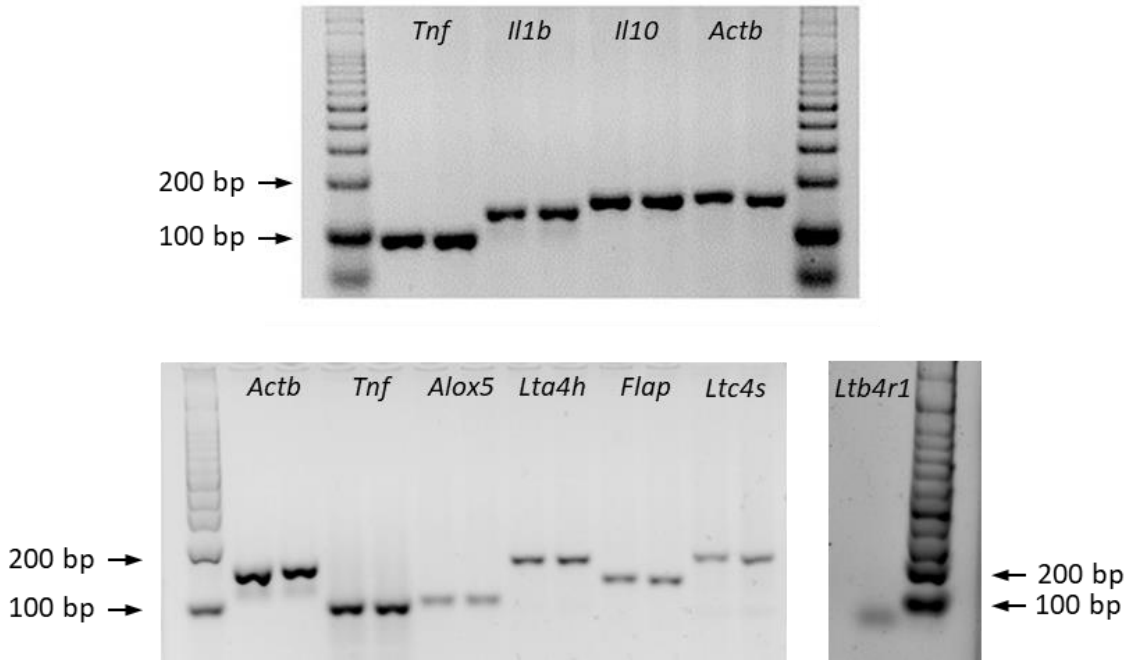

Fig. S1. RT-PCR amplification products. Size-based separation of the PCR products (amplicons) in 3% agarose gel. The products shown were the result of 40 cycles of PCR amplification in samples of liver (top) or Kupffer cells (bottom). For a list of primers and predicted amplicon sizes, see table S1.

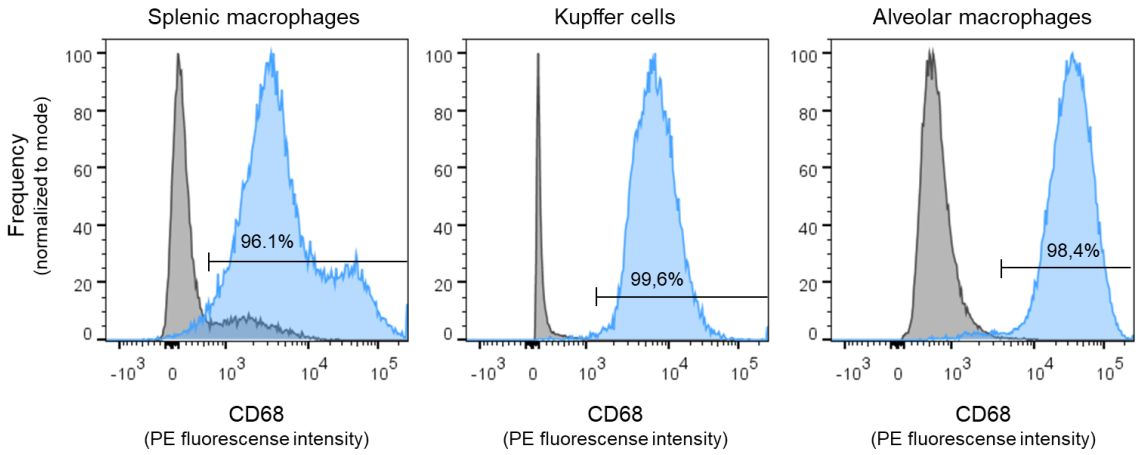

Fig. S2. The purity of resident macrophages in culture was higher than 95%. The figure shows representative flow-cytometry histograms of cells stained (blue) and unstained (gray) for the rat macrophage marker CD68. The cells were obtained at the end of the final incubation period from cultures not stimulated with LPS.

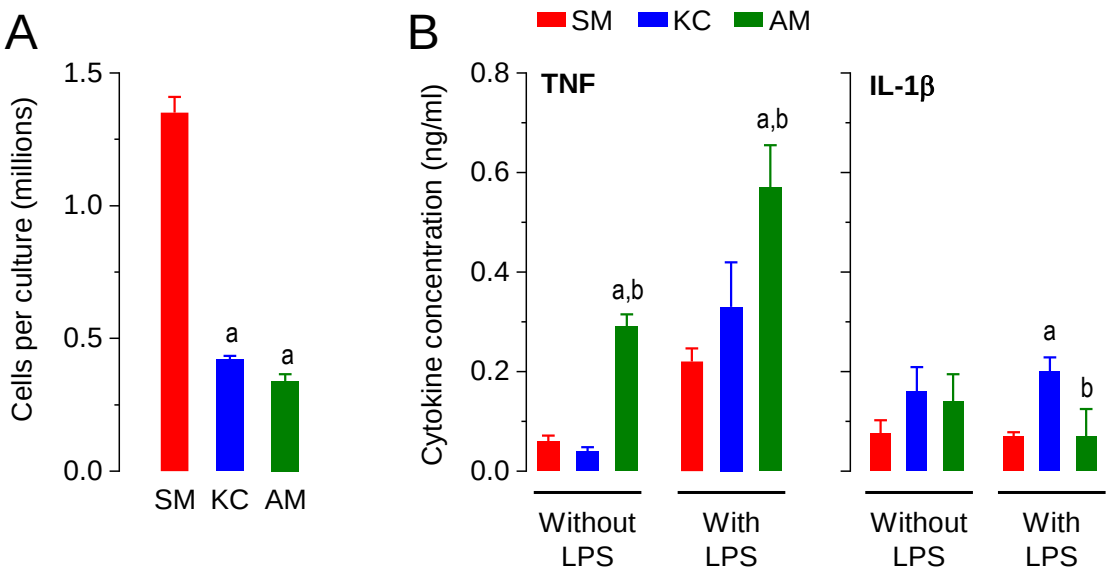

Fig. S3. Cell counts and absolute cytokine concentrations in cultures of spleen, liver, and lung macrophages. (**A)** Splenic macrophages (SM), Kupffer cells (KC), and alveolar macrophages (AM) were isolated from rats. For counting, cells were detached from Cultureware at the time that would otherwise correspond to the beginning of the LPS stimulation period. Cell counts are presented as means ± SEM. The number of cultures in each group was 25 for SM, 27 for KC, and 15 for AM; cells from a single rat yielded 2-4 cultures. (**B)** Concentrations of TNF and IL-1β in the medium of macrophage cultures stimulated or not with LPS for 6 hours. Data are also shown as means ± SEM. Group sizes are 6-16 cultures for SM, 5-9 for KC, and 7-12 for AM. In (A) and (B), a, $p<0.05$ in comparison with SM; b, $p<0.05$ in comparison with KC (ANOVA followed by the Fisher test).

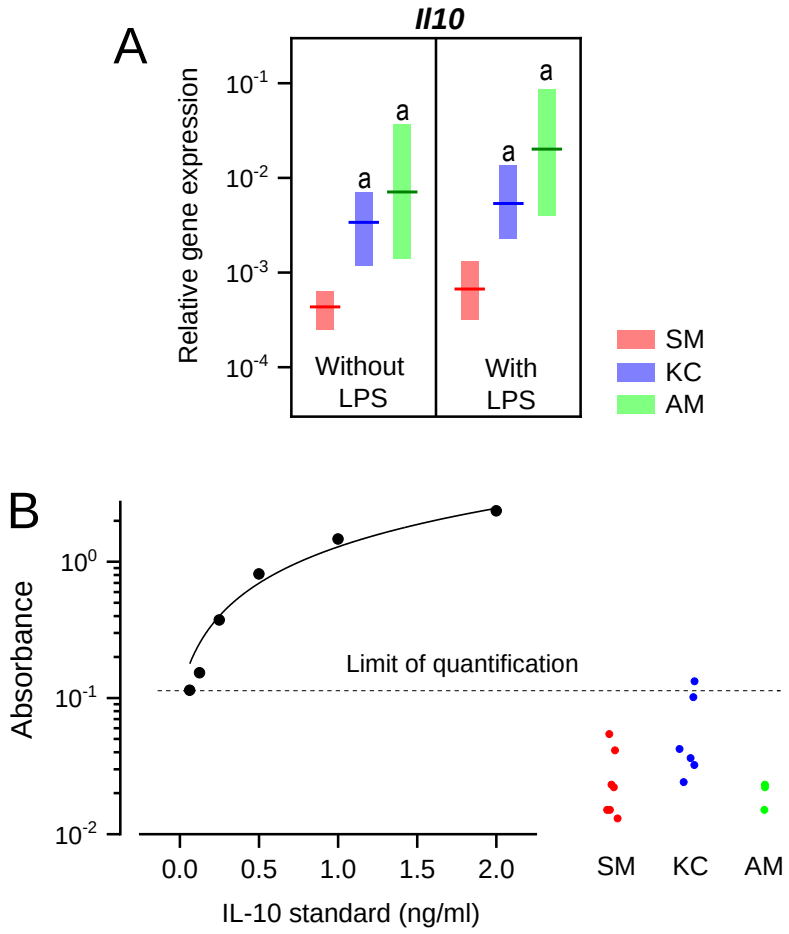

Fig. S4. Expression and secretion of IL-10 in resident macrophages from different organs. (A and B) Splenic macrophages (SM), Kupffer cells (KC), and alveolar macrophages (AM) were isolated from rats and stimulated or not with LPS for 2 hours (A) or 6 hours (B). (**A**) *Il10* expression is shown as median (horizontal line) and 95% confidence intervals (floating bars). The number of cultures in each group was 8 for SM, 4-7 for KC, and 8 for AM; cells from a single rat typically yielded 2-4 cultures, randomly distributed across the group. a, statistically different ($p<0.05$) from SM (Kruskal-Wallis test followed by the Mann-Whitney test). (**B**) Absorbance of standard and samples in a representative IL-10 ELISA assay. With only one exception in a KC culture, all absorbance values obtained for the samples were below the quantification limit of the assay.

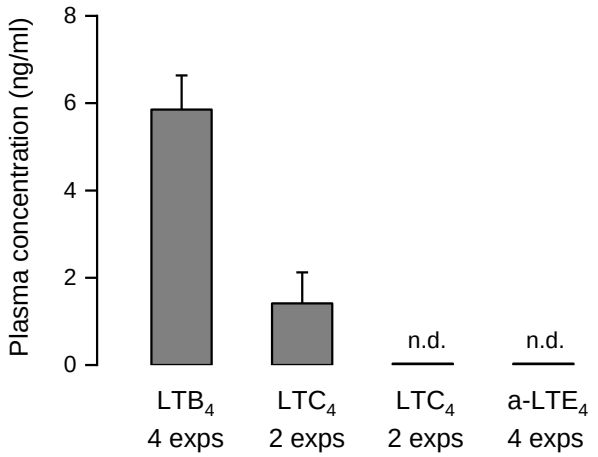

Fig. S5. Comparison of plasma concentrations for LTB_4, LTC_4, and a-LTE_4. The results are representative of four experiments (exps) conducted in LPS-injected rats not subjected to any pharmacological treatment or organ ablation. Samples were collected 80 min after LPS. n > 5 rats for each experiment. Note that LTB_4 was detected in all experiments, whereas LTC_4 was detected in two of the four experiments, and a-LTE_4 (N-acetyl-LTE_4) was never detected. n.d., not detectable.

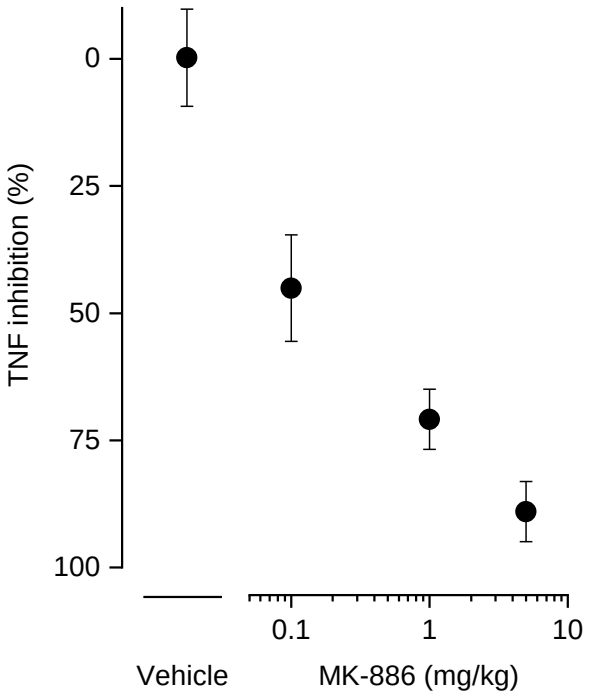

Fig. S6. Dose-dependent effect of MK-886 on LPS-induced plasma concentrations of TNF. Effect of pretreatment with MK-886 (i.p) on the plasma concentration of TNF 80 min after a challenge with LPS (i.v.). The pretreatment was made 90 min before the LPS injection. Data represent the reduction in plasma TNF compared to vehicle-treated control rats stimulated with LPS. The data are shown as means ± SEM of at least 4 samples per dose.

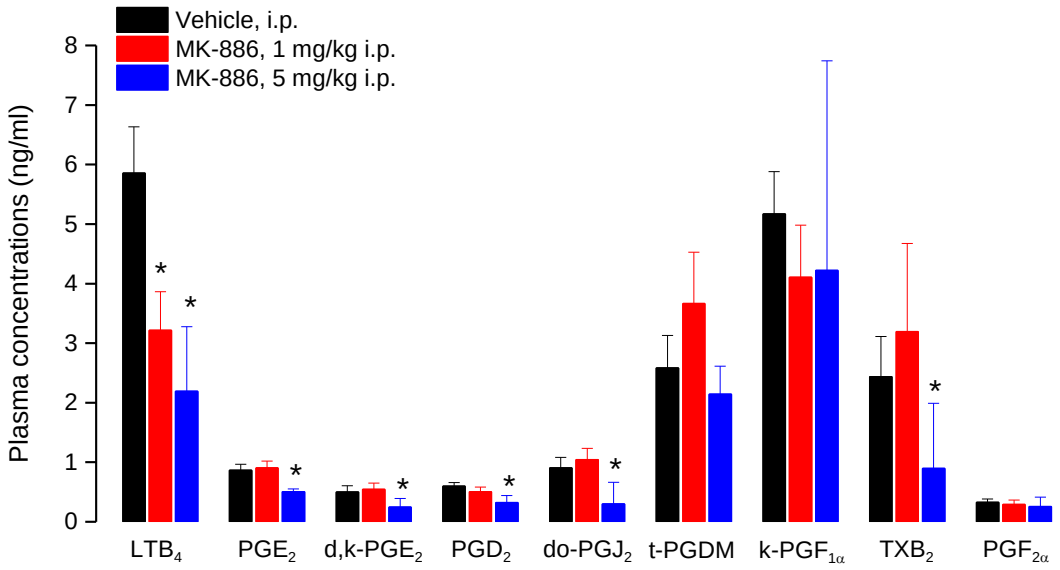

Fig. S7. Plasma concentrations of eicosanoids in LPS-injected rats pretreated with MK-886 or vehicle. Rats were pretreated with the indicated dose of MK-886 or vehicle 90 min before the LPS injection, and samples were harvested at 80 min post-LPS. Data are shown as means ± SEM. Group sizes were of 10 rats for vehicle, 10 rats for the 1-mg/kg dose, and 3 rats for the 5-mg/kg dose. $^{*}p<0.05$ compared to the vehicle-pretreated rats (ANOVA followed by the Fisher test). Abbreviations: LTB_4, leukotriene B_4; PGE_2, prostaglandin E_2; d,k-PGE_2, 13,14-dihydro-15-keto-prostaglandin E_2; PGD_2, prostaglandin D_2; do-PGJ_2, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2; t-PGDM, tetranor-prostaglandin D metabolite; k-$PGF_{1\alpha}$, 6-keto Prostaglandin $F_{1\alpha}$; TXB_2, thromboxane B_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$. Leukotriene C_4 was not detected in this particular experiment.

Table S1. Primers used in quantitative RT-PCR.

Gene name	Accession number	Primers (5' → 3')	Amplicon size (bp)
Tnf	NM_012675.3	CTTCAAGGGACAAGGCTG (forward) GAGGCTGACTTTCTCCTG (reverse)	88
Il1b	NM_031512.2	GAAGTCAAGACCAAAGTGG (forward) TGAAGTCAACTATGTCCCG (reverse)	124
Il10	NM_012854.2	TAAGGGTTACTTGGGTTGCC (forward) TATCCAGAGGGTCTTCAGC (reverse)	142
Alox5	NM_012822.1	CAAGATTGTTCCCATCGCCA (forward) GCCAGTCGTATTTTGAGTCCG (reverse)	89
Flap	NM_017260.2	GGTCTACACTGCCAACCAGA (forward) ATACATCAGCCCAGCGAAGG (reverse)	115
Lta4h	NM_001030031.1	AGATGGGTCACTGCCAAAGAG (forward) TGCATTCGCTTTATGTGCCC (reverse)	143
Ltc4s	NM_053639.2	TCTTCTGGCTACCGTCACCC (forward) ATTTACCTGGGCTCGGAAGA (reverse)	148
Ltb4r1	NM_021656.1	CCAGCTACTCTGACATCGGG (forward) ATAATGAGCACCACCAGGCG (reverse)	85
Ltb4r2	NM_053640	CACGCGGTCAATCTCCTACA (forward) CGGGTTGACGCTGGAACTAA (reverse)	135
Actb	NM_031144.3	GGCATAGAGGTCTTTACGGATG (forward) TCACTATCGGCAATGAGCG (reverse)	143

Table S2. Analytes evaluated by LC-MS/MS.

Analyte	*m/z* transition	Capillary voltage (kV)	Cone voltage (V)	Collision Energy (eV)	Retention time (min)
PGE_2	351.2 > 271.2	2.5	15	16	2.70
d,k-PGE_2	351.2 > 333.2	2.5	20	11	2.72
PGD_2	351.2 > 271.2	2.5	15	16	3.15
t-PGDM	327.2 > 309.2	2.5	25	11	0.75
do-PGJ_2	315.2 > 271.2	2.5	30	14	7.05
k-$PGF_{1\alpha}$	369.2 > 245.2	2.5	30	23	1.27
$PGF_{2\alpha}$	353.2 > 309.2	2.5	20	17	2.37
d,k-$PGF_{2\alpha}$	353.2 > 291.2	2.5	30	21	2.39
TXB_2	369.2 > 169.0	2.5	30	14	1.89
dh-TXB_2	367.2 > 305.2	2.5	30	15	2.88
LTB_4	335.2 > 317.1 335.2 > 195.1 335.2 > 58.9	2.5	30	15 16 23	6.85
LTC_4	625.4 > 272.1 625.4 > 143.1	3.0	15	19	6.77
a-LTE_4	480.3 > 351.2 480.3 > 333.2 480.3 > 115.2	2.5	15	20	6.90
PGE_1 (IS)	353.2 > 317.2	2.5	20	12	2.91

Abbreviations: PGE_2, prostaglandin E_2; d,k-PGE_2, 13,14-dihydro-15-keto-prostaglandin E_2; PGD_2, prostaglandin D_2; t-PGDM, tetranor-prostaglandin D metabolite; do-PGJ_2, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2; k-$PGF_{1\alpha}$, 6-keto Prostaglandin $F_{1\alpha}$; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; d,k-$PGF_{2\alpha}$, 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$; TXB_2, thromboxane B_2; dh-TXB_2, 11-dehydro-thromboxane B_2; LTB_4, leukotriene B_4; LTC_4, leukotriene C_4; a-LTE_4, N-acetyl-LTE_4; PGE_1, prostaglandin E_1; IS, internal standard.

Movie S1. Animated graph of cytokine EMI at 30 min after LPS. The graph is a three-dimensional representation of EMIs for *Tnf*, *Il1b*, and *Il10* in spleen, liver, and lung. Data are shown as median (inner point) and 95% confidence intervals (parametric surface).

Movie S2. Animated graph of cytokine EMI at 80 min after LPS. The graph is a three-dimensional representation of EMIs for *Tnf*, *Il1b*, and *Il10* in spleen, liver, and lung. Data are shown as median (inner point) and 95% confidence intervals (parametric surface).