SUPPLEMENTAL MATERIAL
Supplemental Methods

Chronic angiotensin II and IL-10 infusion

Osmotic minipumps (model 1002, Alzet Durect Corp, USA) containing human Ang II (Millipore-Sigma, USA) were implanted subcutaneously under isoflurane anesthesia. Mice received bupivacaine hydrochloride (Marcaine, CDMV, Canada, 2 mg/kg s.c.) at the site of the incision before and after minipump implantation. Each osmotic pump delivered 1000 ng/kg/min for 14 days. Control animals received a sham surgery. Pilot experiments confirmed no differences between a sham surgery and implantation of a saline-infused minipump for cerebral blood flow analysis. In another group of animals, systemic infusion of 1000 ng of IL-10 was achieved via a second osmotic minipump filled with human recombinant IL-10 (Sigma-Aldrich, USA), delivering IL-10 at a rate of 60 ng/day. IL-10 minipumps were implanted together with the Ang II minipumps on the same day, for continued infusion during 14 days. There were no differential mortality events between the treated and control groups.

In vivo laser Doppler flowmetry

Anesthesia was initiated with isoflurane (induction: 5%, maintenance: 2%) and maintained by intraperitoneal injections of 50 mg/kg of alpha-chloralose (Santa Cruz Biotechnology, USA) and 750 mg/kg of urethane (Sigma-Aldrich, USA). Mean blood pressure and blood gases were monitored via femoral artery catheterization. Ventilation was maintained artificially with a nitrogen/oxygen/CO₂ mixture through a tracheal intubation. Body temperature was maintained at 37 °C. Cerebral blood flow (CBF) was monitored by a laser Doppler probe (AD Instruments, USA) placed in a 2x2 mm cranial window drilled above the somatosensory cortex. Artificial cerebrospinal fluid (aCSF, NaCl 125 mM, KCl 3 mM, NaHCO₃ 26 mM, NaH₂PO₄H₂O 1.25 mM, CaCl₂ 2 mM, MgCl₂6H₂O 1mM, glucose 4 mM, ascorbic acid 0.4 mM, bubbled with 95% O₂, 5% CO₂ for 10 minutes) was superfused (0.5 mL/min, 35 °C) after the removal of the pia mater. Analysis of CBF responses began 30 minutes after the end of the surgery to allow blood gases to stabilize. Animals with a mean arterial blood pressure under 60 mmHg and/or blood gases outside normal range (pH: 7.35-7.40; pCO₂: 33-45; pO₂: 120-140) were eliminated from the study. This comprised 5 animals for the CBF experiments with wild-type CD4⁺CD25⁺ cells, 1 animal for the CBF experiment with the CD4⁺CD25⁺ Il10⁻/⁻ cells and 6 animals for the CBF experiment with IL-10. The laser Doppler probe was placed stereotaxically above the whisker barrel area at the site of superfusion. CBF responses to neuronal activity were examined by whisker stimulation (three 1-minute stimulations at 6 Hz every 3 minutes on the contralateral side to CBF measurement). Endothelium-dependent CBF responses were measured after the superfusion of acetylcholine 10 μM (Sigma-Aldrich, USA), superfused for 5 minutes followed by a 15 minutes aCSF superfusion to restore basal stimulation level. CBF values were acquired with the LabChart6 Pro software (v6.1.3, AD Instruments, USA). The percentage increase in CBF was measured using the CBF values before the stimulations and the maximum response.
**Blood pressure**

Systolic blood pressure was monitored by non-invasive tail-cuff plethysmography (Kent Scientific Corp., USA). The measures were taken 24 hours before analysis of cerebral blood flow. Mice were warmed on a heating pad preheated at 37 °C for 10 minutes before and during blood pressure recordings. Animals were habituated to the procedure the three days prior to blood pressure assessment. On the experimental day, following stabilization, ten blood pressure measurements per mouse were done and averaged for analysis. Data were not considered valid if the blood flow in the tail was below 15 µL.

**Plasma cytokine/chemokine array and composite score calculation**

Blood was collected via cardiac puncture in heparinized tubes. Samples were centrifuged at 2000 g for 20 minutes at 4°C to separate plasma. Cleared plasma samples were stored at −80°C and were only thawed for cytokine/chemokine analysis. A multiplex bead-based immunoassay from Eve Technologies Corporation (Calgary, AB, Canada) was used for the quantitative determination of 31 mouse plasma cytokines and chemokines (MD31 Discovery Assay, Mouse Cytokine/Chemokine Array 31-Plex). Plasma samples were diluted in half in PBS pH 7.4 and shipped on dry ice for analysis. The array measured the levels of Eotaxin (CCL11), G-CSF, GM-CSF, IFNγ, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-15, IL-17A, IP-10 (CXCL10), KC (CXCL1), LIF, LIX (CXCL5), MCP-1 (CCL2), M-CSF, MIG (CXCL9), MIP-1α, MIP-1β (CCL4), MIP-2 (CCL2), RANTES (CCL5), TNFα and VEGF. Analyte concentrations were expressed in pg/mL and calculated through a standard curve from fluorescence intensity values. The following were not included in the final analysis due to low or no detection in several samples: IL-3, IL-7, IFNγ, IL-1β, GM-CSF, M-CSF, VEGF and MIP-1α. Given the large number of cytokines/chemokines analyzed a composite inflammatory Z score was computed to obtain a global measure reflecting inflammation and providing a more powered analysis. Prior to composite score calculation and in consultation with an immunologist (Dr. Jean-François Gauchat, Université de Montréal, Canada), cytokines and chemokines were grouped into four categories according to their main function/immune effect: I) Pro-inflammatory cytokines: IL-1α, IL-6, IL-17, TNF-α and LIF; II) Neutrophil chemoattractants: KC (CXCL1), LIX (CXCL5), MIP-2 (CXCL2) and stimulators of their development (G-CSF); III) Stimulators of Th1-driven responses: IL-12p40, IL12p70, MIP-1β (CCL4), RANTES (CCL5), MIG (CXCL9) and IP-10 (CXCL10); IV) Stimulators of Th2-driven responses: IL-4, IL-5, IL-9, IL-10, IL-13 and MCP-1 (CCL2). Eotaxin (CCL11) and IL-2 were not classified in any of the groups and are presented independently. The grouping does not intend to reflect the cellular source of each cytokine but rather their main action/effect as immune mediators. Within each group a cytokine/chemokine composite score was calculated for each mouse by converting each independent marker to a standardized Z score, such that the group mean was zero and the standard deviation was 1. Z scores were obtained with the formula $z = (x - \mu) / \sigma$, where $x$ was the individual value of the marker to be standardized, $\mu$ the mean of the dataset for that marker and $\sigma$ the standard deviation. To generate the composite, Z scores within a group were added.
**Microglia immunohistochemistry analysis**

PFA-fixed brain sections (40 μm thick) were incubated in 0.3% hydrogen peroxide (Sigma-Aldrich, USA) for 20 minutes followed by 3 washes in PBS and 1 wash in PBS-T (PBS + 0.2% Triton X-100). Blocking was done with 10% normal goat serum in PBS-T during 1 hour at room temperature. Microglia/monocytes were labeled with Iba-1 (1:2000, Wako Inc, Richmond, USA) in PBS-T with 10% goat serum, overnight at 4 °C. The following day, sections were washed in PBS-T and incubated in biotinylated goat anti-rabbit antibody (ABC Kit, Vector) at 1:500 in PBS-T with 5% goat serum for 1 h. Signal amplification was done following manufacturer’s instructions. Immunoreactions were developed with 0.06% 3,3’-diaminobenzidine and 1% hydrogen peroxide (Sigma-Aldrich, USA) in PBS. Following mounting, sections were dehydrated and defatted in increasing ethanol concentrations and xylenes, then coverslipped. Bright field images were taken with a Leitz Diaplan microscope equipped with an Olympus DP21 camera (Wild Leitz GmbH, Germany). Images were taken from layer V-VI of the somatosensory cortex (8 pictures per brain section) and from the hippocampal regions CA1, CA3 and DG (2 pictures per region per brain section). Micrographs were imported into the ImageJ software for calculation of microglial cell number. Each 8-bit image was thresholded with the intermodes method and converted to binary. The ‘Analyze Particles’ function was used to obtain the approximate number of microglia in the whole micrograph, using a size criteria of 150 pixels. Three different brain sections per animal were examined, and an average per region was calculated per section. Each was used for analysis.

**Superoxide anion production**

Brain cortical superoxide anion production was measured by lucigenin-enhanced chemiluminescence using a scintillation counter (Wallac 1409, Perkin Elmer, Canada) in out-of-coincidence mode with a single active photomultiplier tube as previously described (Sadokova et al, JAHA 2013). Cortical tissue was incubated at 37°C for 15 minutes in an oxygenated Krebs-HEPES buffer in the presence of 100 μmol/L NADPH (Sigma-Aldrich, Canada). Following the addition of lucigenin (5 μmol/L, Sigma-Aldrich, Canada) counts were obtained at 1-minute intervals during 10 minutes and corrected for background (reaction done without brain tissue). Superoxide production was determined based on the area under the curve (AUC) of counts/time. A second cortical sample was first incubated with the selective NOX-2 inhibitor, gp91ds-tat (50 μmol/L, AnaSpec, USA), in Krebs-HEPES buffer during 45 minutes, followed by the addition of NADPH and lucigenin. Counts were obtained as described above. Given that lucigenin detects superoxide anions from various enzymatic oxidase systems, NOX-2-derived superoxide production was expressed as the percentage of inhibition by gp91ds-tat relative to the amount of superoxide produced without the inhibitor. Each value was normalized to tissue weight.
Table S1. Description of antibodies used for flow cytometry analysis.

| Antigen | Fluorochrome | Clone  | Manufacturer                      |
|---------|--------------|--------|-----------------------------------|
| CD3     | BV737        | 17A2   | BD Biosciences                    |
| CD11b   | eFluor 450   | M1/70  | eBioscience ThermoFisher Scientific |
| F4/80   | PeCy7        | BM8    | eBioscience ThermoFisher Scientific |
| CD11c   | PerpCy5.5    | HL3    | BD Biosciences                    |
| CD19    | APC          | eBio1D3| eBioscience ThermoFisher Scientific |
| Ly6G    | Alexa700     | 1A8    | Biolegend                         |
| CD45.2  | APC-eFluor 780| 104    | eBioscience ThermoFisher Scientific |
| CD49b   | PE           | DX5    | eBioscience ThermoFisher Scientific |
| Ki67    | BUV395       | B56    | BD Biosciences                    |
| CD8a    | PECy7        | 53-6.7 | eBioscience ThermoFisher Scientific |
| CD4     | Alexa700     | GK1.5  | eBioscience ThermoFisher Scientific |
| CD45.1  | PerpCy5.5    | A20    | eBioscience ThermoFisher Scientific |
| CD25    | APC          | PC61.6 | eBioscience ThermoFisher Scientific |
| CD3e    | eFluor 450   | 17A2   | eBioscience ThermoFisher Scientific |
| Helios   | PE           | 22F6   | BD Biosciences                    |
Table S2. Effect of CD4\(^{+}\)CD25\(^{+}\) T lymphocytes on systolic blood pressure.

| SBP (mmHg) | PBS   | CD4\(^{+}\)CD25\(^{+}\) | CD4\(^{+}\)CD25\(^{+}\) Il10\(^{-/-}\) |
|------------|-------|--------------------------|----------------------------------|
| CTL        | 124.0 ± 2.5 | 129.3 ± 5.3 | 121.7 ± 9.9 |
| Ang II     | 175.5 ± 6.0\(^{III}\) | 177.4 ± 6.9\(^{I}\) | 183.3 ± 6.3\(^{III}\) |

Systolic blood pressure (SBP) was measured by tail-cuff plethysmography following 14 days of Ang II administration (1000 ng/kg/min) or a sham surgery for controls (CTL). Data is expressed as mean ± SEM, \(n=6-10\)/group; analyzed by two-way ANOVA, interaction: F(2,37) = 0.539 \(p=0.589\); Ang II effect: F(1,37) = 96.43 \(p<0.0001\); effect of CD4\(^{+}\)CD25\(^{+}\) cells: F(2,37) = 0.167, \(p=0.847\); \(^{I}\) \(p<0.001\), \(^{III}\) \(p<0.0001\) Ang II versus CTL.
Table S3. Effect of exogenous IL-10 administration on systolic blood pressure.

| SBP (mmHg) | PBS   | IL-10 |
|------------|-------|-------|
| CTL        | 135.0 ± 1.7 | 132.8 ± 2.0 |
| Ang II     | 172.3 ± 8.6$^\parallel$ | 169.7 ± 2.3$^\parallel$ |

Systolic blood pressure (SBP) was measured by tail-cuff plethysmography following 14 days of Ang II administration (1000 ng/kg/min) or Ang II + IL-10 (60 ng/day) or a sham surgery for controls (CTL). Data is expressed as mean ± SEM, $n=3-6$ group; analyzed by two-way ANOVA, interaction: $F(1,11) = 0.004, p=0.952$; Ang II effect: $F(1,11) = 83.63, p<0.0001$; effect of IL-10: $F(1,11) = 0.355, p=0.563$; $\parallel p<0.001$ Ang II versus CTL.
Figure S1. Effect of CD4⁺CD25⁺ adoptive transfer on plasma levels of pro-inflammatory cytokines induced by Ang II. The plasma levels (pg/ml) of individual pro-inflammatory cytokines were examined with a multiplex bead-based immunoassay following adoptive transfer of CD4⁺CD25⁺ or CD4⁺CD25⁺ IL10⁻/⁻ cells or PBS in mice infused s.c. with Ang II (1000 ng/kg/min, 14 days) or in mice that received a sham surgery (Sham). Graphs represent mean ± SEM (n=4-11/group, *p<0.05 and #p<0.05 by one-way ANOVA followed by Bonferroni correction).
Figure S2. Effect of CD4^+CD25^+ adoptive transfer on plasma levels of neutrophil chemoattractants and stimulators of their development induced by Ang II. The plasma levels (pg/ml) of neutrophil chemoattractants (KC/CXCL1, MIP-2/CXCL2 and LIX/CXCL5) and stimulators of their development (G-CSF) were examined with a multiplex bead-based immunoassay following adoptive transfer of CD4^+CD25^+ or CD4^+CD25^+ Il10^-/- cells or PBS in mice infused s.c. with Ang II (1000 ng/kg/min, 14 days) or in mice that received a sham surgery (Sham). Graphs represent mean ± SEM (n=4-11/group, *p<0.05, by one-way ANOVA followed by Bonferroni correction).
Figure S3. Effect of CD4^+CD25^+ adoptive transfer on plasma levels of stimulators of Th1-driven responses induced by Ang II. The plasma levels (pg/ml) of individual cytokines (IL-12p40 and IL-12p70) and chemokines (MIP-1β/CCL4, RANTES/CCL5, MIG/CCL9 and IP-10/CXCL10) involved in Th1 stimulation were examined with a multiplex bead-based immunoassay following adoptive transfer of CD4^+CD25^+ or CD4^+CD25^- Il10^−/− cells or PBS in mice infused s.c. with Ang II (1000 ng/kg/min, 14 days) or in mice that received a sham surgery (Sham). Graphs represent mean ± SEM (n=4-11/group, *p<0.05, by one-way ANOVA followed by Bonferroni correction).
Figure S4. Effect of CD4⁺CD25⁺ adoptive transfer on plasma levels of stimulators of Th2-driven responses. The plasma levels (pg/ml) of cytokines (IL-4, IL-5, IL-9, IL-10 and IL-13) and chemokines (MCP-1/CCL2) involved in stimulation of Th2 responses were examined with a multiplex bead-based immunoassay following adoptive transfer of CD4⁺CD25⁺ or CD4⁺CD25⁺ IL10⁻⁺ cells or PBS in mice infused s.c. with Ang II (1000 ng/kg/min, 14 days) or in mice that received a sham surgery (Sham). Graphs represent mean ± SEM (n=4-11/group, no significant differences between groups by one-way ANOVA followed by Bonferroni correction).
Figure S5. Effect of CD4⁺CD25⁺ adoptive transfer on plasma levels of eotaxin and IL-2 induced by Ang II. The plasma levels (pg/ml) of eotaxin (CCL11) and IL-2 were examined with a multiplex bead-based immunoassay following adoptive transfer of CD4⁺CD25⁺ or CD4⁺CD25⁺ Il10⁻/⁻ cells or PBS in mice infused s.c. with Ang II (1000 ng/kg/min, 14 days) or in mice that received a sham surgery (Sham). Graphs represent mean ± SEM (n=4-11/group, no significant differences between groups by one-way ANOVA followed by Bonferroni correction).
Figure S6. Effect of CD4⁺CD25⁺ II10⁻ adaptive transfer on plasma levels of pro-inflammatory cytokines in normal mice. The plasma levels (pg/ml) of pro-inflammatory cytokines were examined with a multiplex bead-based immunoassay following adoptive transfer of CD4⁺CD25⁺, CD4⁺CD25⁺ II10⁻ cells or PBS in C57BL/6 mice. Graphs represent mean ± SEM (n=4-11/group, *p<0.05, by one-way ANOVA followed by Bonferroni correction).
Figure S7. Effect of CD4+CD25+ Il10−/− adoptive transfer on plasma levels of stimulators of Th1-driven responses in normal mice. The plasma levels (pg/ml) of cytokines (IL-12p40 and IL-12p70) and chemokines (MIP-1β/CCL4, RANTES/CCL5, MIG/CCL9 and IP-10/CXCL10) involved in Th1 stimulation were examined with a multiplex bead-based immunoassay following adoptive transfer of CD4+CD25+, CD4+CD25+ Il10−/− cells or PBS in C57BL/6 mice. Graphs represent mean ± SEM (n=4-11/group, *p<0.05, ***p<0.001, # p<0.05 by one-way ANOVA followed by Bonferroni correction).
Figure S8. Effect of CD4⁺CD25⁺ II10⁻⁻ adoptive transfer on plasma levels of neutrophil chemoattractants and stimulators of their development in normal mice. The plasma levels (pg/ml) of neutrophil chemoattractants (KC/CXCL1, MIP-2/CXCL2 and LIX/CXCL5) and stimulators of their development (G-CSF) were examined with a multiplex bead-based immunoassay following adoptive transfer of CD4⁺CD25⁺, CD4⁺CD25⁺ II10⁻⁻ cells or PBS in C57BL/6 mice. Graphs represent mean ± SEM (n=4-11/group, *p<0.05 by one-way ANOVA followed by Bonferroni correction).
**Figure S9.** Effect of CD4⁺CD25⁺ Il10⁻/⁻ adoptive transfer on plasma levels of cytokines (IL-4, IL-5, IL-9, IL-10 and IL-13) and chemokines (MCP-1/CCL2) involved in Th2 stimulation were examined with a multiplex bead-based immunoassay following adoptive transfer of CD4⁺CD25⁺, CD4⁺CD25⁺ Il10⁻/⁻ cells or PBS in C57BL/6 mice. Graphs represent mean ± SEM (n=4-11/group, *p<0.05, by one-way ANOVA followed by Bonferroni correction).
Figure S10. Analysis of Treg distribution. A) Flow cytometry analysis of the expression of CD8, CD4, Foxp3-GFP and CD25 from purified CD25^+ cells isolated from the spleen of C57BL/6 Foxp3<sup>GFP</sup> CD45.1^+ male mice by magnetic bead cell sorting (MACs® Miltenyi Biotec, Bergisch Gladbach, Germany) at the time of transfer (similar purity both at day 1 and day 7 of transfer); B) Analysis of cell suspensions of CD45.1^+ exogenous Treg isolated from inguinal lymph nodes (pLN), spleen, and PBS-perfused brain and kidney dissected from C57BL/6 mice that received adoptive transfer of CD4^+CD25^+ cells (3x10^5) and were infused s.c. with Ang II (1000 ng/kg/min, 14 days) (Ang II + CD4^+CD25^+) or from mice that received a sham surgery (CTL+ CD4^+CD25^+).
Figure S11. Analysis of immune cell populations in brain.
A) Flow cytometry analysis of the cerebral expression of neutrophils/granulocytes (N/Gs) (CD45.2+Ly6G+CD11b+), dendritic cells (DCs) (CD45.2-Cd11b-Cd11c-Cd19+), B cells (CD45.2+CD11b+CD11c+CD19+), CD3 (CD45.2+, CD3+CD19+ CD49b+) and natural killer (NK) cells (CD45.2+CD11c+CD11b- CD3 CD49b+) in C57BL/6 mice that received adoptive transfer of CD4+CD25+ cells (3x10^6) and were infused s.c. with Ang II (1000 ng/kg/min, 14 days) (Ang II + CD4+CD25+) or in mice that received a sham surgery (CTL+ CD4+CD25+). B) Gating strategy for myeloid cell determination used by flow cytometry in C57BL/6 mice that received adoptive transfer of CD4+CD25+ cells (3x10^6) and that were infused with Ang II (1000 ng/kg/min, 14 days) (Ang II + CD4+CD25+) or in mice that received a sham surgery (CTL+ CD4+CD25+).