Supporting Information

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SI Materials and Methods

Lung Single Cell Preparation, Flow Cytometry, and Th2 Cytokine ELISAs. Lungs were dissected, enzymatically digested with 0.5 mg/mL collagenase type IA (Sigma-Aldrich) and pushed through a 70-μm nylon cell strainer. The antibodies used for staining were anti-MHCII (clone M5/114.15.2), anti-B220 (RA3-6B2), anti-CD11c (clone HL3), anti-CD103 (clone M290), and anti-CD11b (clone M1/70; all BD Pharmingen). FACS analyses were performed on a FACSCanto2 cytometer (BD Biosciences); post-acquisition analysis was done using FlowJo software (Tree Star). Cytokines in lung single-cell cultures restimulated for 72 h with 250 μg/mL ovalbumin were quantified by ELISA (IL-5, BD Pharmingen; IL-13, R&D Systems).

Preparation of Murine and Human DCs and IL-10 ELISA. For generation of murine bone-marrow-derived dendritic cells (BM-DCs), bone marrow isolated from the hind legs of donor mice (BL/6.TLR2$^{-/-}$, BL/6.TLR4$^{-/-}$, BL/6.MyD88$^{-/-}$ mice, all from Jackson Labs) was seeded at 50,000 cells per well in 96-well plates in RPMI/10% (vol/vol) FCS and 4 ng/mL GM-CSF and cultured for 5 d. For the isolation of MLN-DCs, mesenteric lymph nodes were digested in 1 mg/mL collagenase (Sigma-Aldrich) for 30 min at 37 °C with shaking before filtering through a cell strainer (40 μm; BD Biosciences) and immunomagnetic isolation of DCs using mouse-specific CD11c microbeads (Miltenyi Biotec). BM-DCs and MLN-DCs were stimulated with the indicated amounts of *Helicobacter pylori* PMSS1 extract for 16 h, and supernatants were subjected to mIL-10 ELISA (BD Pharmingen). Human monocyte-derived dendritic cells were generated from peripheral blood mononuclear cells. Venous blood was drawn from six healthy volunteers according to protocols approved by the Institutional Review Board of Leiden University Medical Center. Cells were collected after density gradient centrifugation on Ficoll, and CD14^{+} monocytes were positively isolated by magnetic-activated cell sorting (MACS) using CD14 microbeads (Miltenyi Biotec). Cells were cultured in RPMI-1640 (Invitrogen) supplemented with penicillin (100 U/mL, Astellas Pharma), streptomycin (100 μg/mL, Sigma), pyruvate (1 mM, Sigma), glutamate (2 mM, Sigma), 10% FCS, 20 ng/mL human recombinant granulocyte-macrophage colony-stimulating factor (rGM-CSF, Invitrogen/Life Technologies), and 0.86 ng/mL human rIL-4 (R&D Systems) for 6 d. On day 3, the medium and the supplements were refreshed. Monocyte-derived DCs were stimulated with *H. pylori* extract for 48 h. Secretion of IL-10 by the DCs in the supernatant was measured by ELISA (Sanquin).

DC/T-Cell Cocultures. For Treg differentiation ex vivo, BM-DC cultures were infected overnight with wild-type *H. pylori* PMSS1 or treated with 25 μg/mL *H. pylori* extract. Bacteria were killed with 200 U penicillin/0.2 mg streptomycin/mL for 6 h before the addition of T cells. CD4^{+}CD25^{-} T cells were prepared from single-cell suspensions of naive C57BL/6 spleens by immunomagnetic sorting (R&D Systems). DCs were cocultured with CD4^{+}CD25^{-} T cells at a ratio of 1:2 (0.5×10^5 DC to 1×10^5 T cells) in RPMI containing 10% FCS, 10 ng/mL rTGF-β (PeproTech), 10 ng/mL rIL-2 (R&D Systems) and 1 μg/mL anti-CD3ε (BD Bioscience). After 72 h of coculture, the cells were stained first for CD4 and CD25 and then, after fixation and permeabilization, for FoxP3 (FoxP3-APC, eBioscience). The percentage of FoxP3^{+} CD4^{+} T cells was assessed by FACS on a Cyan ADP 9 instrument (Beckman Coulter) and analyzed using FlowJo software (TreeStar).

Processing of Gastric Tissue for Plating and Colony Counting. Stomachs were retrieved and dissected longitudinally. For the quantitative assessment of *H. pylori* colonization, a stomach section containing representative amounts of antral and corpus tissue was homogenized in *Brucella* broth and serial dilutions were plated on horse blood plates for colony counting as described (1).

1. Arnold IC, et al. (2011) Tolerance rather than immunity protects from *Helicobacter pylori*-induced gastric preneoplasia. *Gastroenterology* 140(1):199–209.

Fig. S1. The protection against asthma conferred by treatment with whole cell extract is specific to *H. pylori*, depends on a heat-sensitive component of the bacteria, and is most efficient when initiated in newborn mice. (*A–E*) Mice were sensitized i.p. with alum-adjuvanted ovalbumin at 8 and 10 wk of age and challenged with aerosolized ovalbumin 2 wk after the second sensitization to induce asthma-like symptoms. Mock-sensitized mice served as negative controls. One group received once-weekly doses of 200 μg of *H. pylori* extract from day 7 of age until the second sensitization. (*A–D*) Airway hyperresponsiveness in response to the indicated increasing doses of methacholine. (*E*) Ovalbumin-specific serum IgE titers of the groups shown in *A–D*. (*F–I*) Mice were treated as described in *A–E*, with the following modifications: Hp_{neo}, extract administered orally from day 7 to the second sensitization, exactly as described above; $Hp_{i.p.}$, extract administered i.p. from day 7 to the second sensitization; Hp_{ad}, extract administered orally to adult mice for 4 wk before the second sensitization; Hp_{4x}, extract administered four times orally in the first 3 wk of life only; $Hp_{h.i.}$, heat-inactivated extract administered orally from day 7 to the second sensitization; $St_{neo/ad}$, Ec_{neo}, *Salmonella typhimurium* and *Escherichia coli* extract administered orally either beginning in the neonatal period or to adults, respectively. (*F* and *G*) Total cells and eosinophils contained in 1 mL of BALF. (*H* and *I*) Tissue inflammation and goblet cell metaplasia. Data points are pooled from two independent studies.

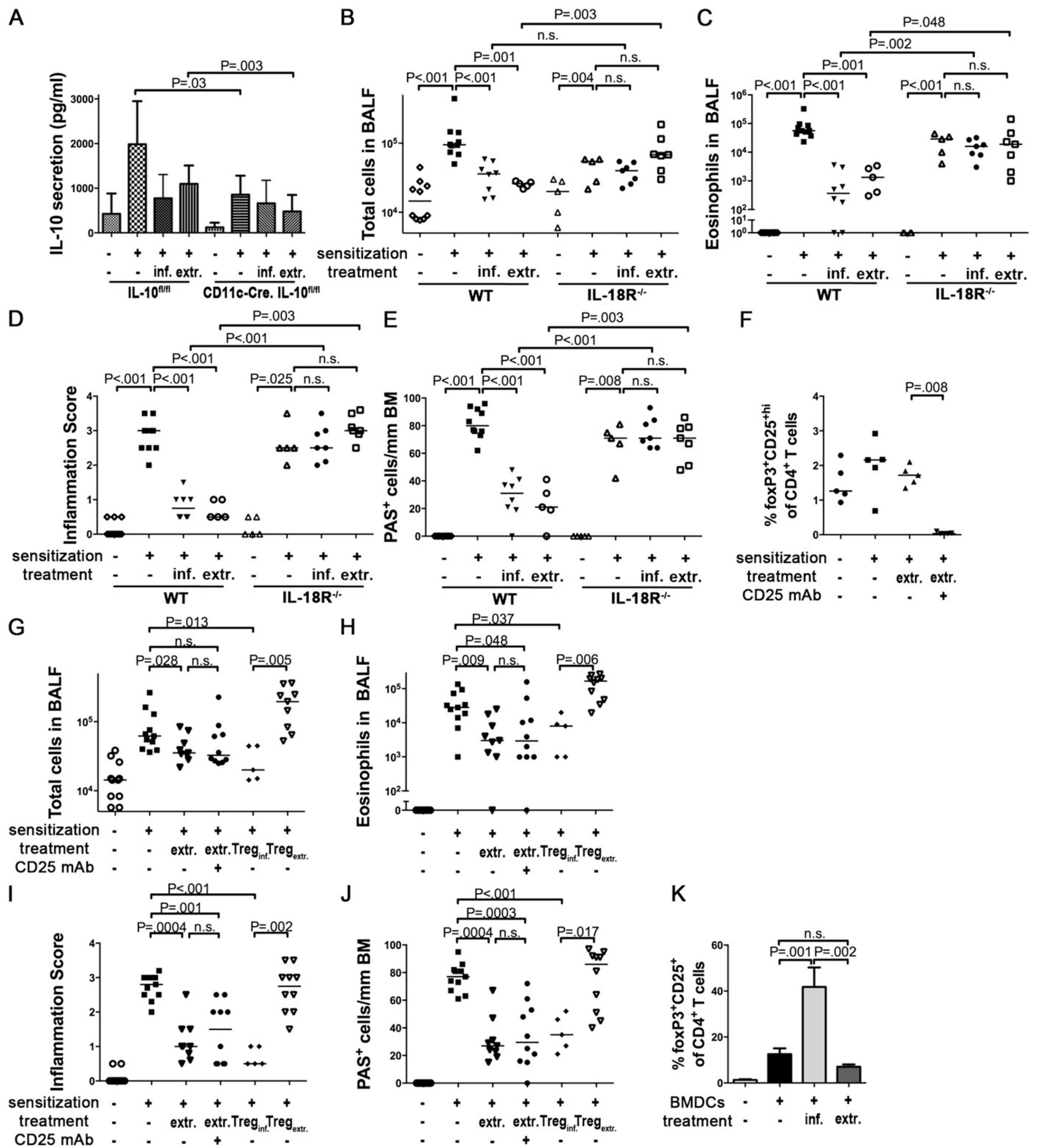

Fig. S2. The protection against asthma conferred by *H. pylori* depends on IL-10 and IL-18, but not on regulatory T cells. (*A*) CD11c-Cre.IL-$10^{fl/fl}$ mice and their IL-$10^{fl/fl}$ littermates were treated with *H. pylori* extract before being subjected to ovalbumin sensitization and challenge as described in Fig. 2 *G–J*. IL-10 secretion by single cell lung preparations restimulated with ovalbumin, as assessed by ELISA. (*B–E*) WT C57BL/6 and IL-$18R^{-/-}$ mice were neonatally infected with *H. pylori* or treated with *H. pylori* extract before being subjected to ovalbumin sensitization and challenge as described in Fig. 1. (*B* and *C*) Total cells and eosinophils contained in 1 mL of BALF. (*D* and *E*) Tissue inflammation and goblet cell metaplasia. (*F–J*) Wild-type C57BL/6 mice were treated with *H. pylori* extract before being subjected to ovalbumin sensitization and challenge. One group received two doses of anti-CD25 antibody during ovalbumin challenge. Additional sensitized and challenged groups received 100,000 immunomagnetically isolated $CD4^+CD25^+$ T cells from either neonatally infected donors or extract-treated donors i.v. 2 d before the first ovalbumin challenge. (*F*) Lung infiltration of $CD4^+CD25^+FoxP3^+$ T cells as assessed in lung single cell preparations of individual mice. (*G* and *H*) Total cells and eosinophils contained in 1 mL of BALF. (*I* and *J*) Tissue inflammation and goblet cell metaplasia. (*K*) BM-DCs were either infected overnight with live *H. pylori* or treated with 25 μg/mL *H. pylori* extract. Bacteria were killed with antibiotics before the addition of splenic $CD4^+CD25^-$ T cells at a DC:T-cell ratio of 1:2. DC/T-cell cocultures were supplemented with rTGF-β, rIL-2and anti-CD3ε. After 72 h of coculture, the cells were stained for CD4, CD25, and FoxP3 and the fraction of $FoxP3^+$ $CD4^+$ T cells was assessed by FACS.

Fig. S3. CD103⁺ conventional DCs accumulate in the lungs of *H. pylori*-infected and extract-treated wild-type mice, but not basic leucine zipper ATF-like 3 (BATF3)$^{-/-}$ mice. (*A–D*) Total cells and eosinophils contained in 1 mL of BALF, as well as tissue inflammation and goblet cell metaplasia as assessed on H&E and PAS-stained tissue sections, of all mice for which lung DC populations are shown in Fig. 3 *A–G*. (*E*) Plasmacytoid DCs (pDCs) and conventional DCs (cDCs) differentially express B220; a representative scatter plot is shown for all CD11c^{+} MHCII^{+} DCs of an extract-treated mouse. (*F*) Total numbers of B220^{+} pDCs infiltrating the lungs of the mice shown in Fig. 3 *A–G*. (*G*) Two distinct cDC lineages can be discriminated in the lung based on CD103 and CD11b expression; a representative scatter plot is shown for an extract-treated mouse (gated on CD11c^{+} MHCII^{+} B220^{-} DCs). (*H*) Total CD103^{+} cDC infiltration into the lungs of the mice shown in Fig. 3 *A–G*. (*I* and *J*) BATF3$^{-/-}$ mice lack CD103^{+} cDCs, but retain CD11b^{+} cDCs and normal frequencies of pDCs. (*K*) *H. pylori* colonization of neonatally infected WT C57BL/6 and BATF3$^{-/-}$ mice as determined by plating of gastric mucosal homogenates on horse blood plates and colony counting.

Fig. S4. VacA protects against allergic asthma when administered intraperitoneally or intragastrically. (*A–E*) Mice were i.p. injected weekly with 25 μg per dose of either recombinant GGT or purified wild-type or mutant (Δ6–27) VacA starting on day 7 of age until the second sensitization as described in Fig. 4 *E–H* (a subset of the mice of Fig. 4 *E–H* is shown here). (*A* and *B*) IL-13 and IL-5 secretion, as assessed by ELISA, of single cell lung preparations restimulated with ovalbumin. (*C* and *D*) Frequencies of $CD103^+$ and $CD11b^+$ cells among all $CD11c^+MHC^+B220^-$ cDCs infiltrating the lungs of the indicated groups of mice. (*E*) Ratios of $CD11b^+$ and $CD103^+$ cDCs as calculated per mouse. (*F* and *G*) Purified wild-type VacA was either administered intraperitoneally (i.p.) or intragastrically (p.o.) as indicated. Mice received either 5 μg or 20 μg of VacA (as indicated), either from age day 7 onwards until 2 wk before challenge (as indicated by subscript "a") or three doses only (delivered in weeks 1, 2, and 3 of life, denoted by subscript "b") or as adults (denoted by subscript "c"). Two doses of IL-10R blocking antibody were administered during challenge where indicated. (*F* and *G*) Total cells and eosinophils contained in 1 mL of BALF. (*H* and *I*) Tissue inflammation and goblet cell metaplasia. Three doses of VacA delivered before weaning were insufficient to provide full protection ("b"). Treatment of adults was almost as protective as neonatal-onset treatment (compare "c" and "a"; note that the total cell count in the adult-treated group is inconsistent with the other three readouts for reasons that are not clear); the 5-μg and 20-μg doses provided similar levels of protection; blocking IL-10 signaling abrogated protection.