

LEAD THE RACE

Comprehensive **flow cytometry** solutions designed to accelerate your work



Learn More

MILIPORE
SIGMA



Therapeutic Application of an Extract of *Helicobacter pylori* Ameliorates the Development of Allergic Airway Disease

This information is current as of January 22, 2018.

Yolanda van Wijck, Stan de Kleijn, Gerrit John-Schuster, Tinne C. J. Mertens, Pieter S. Hiemstra, Anne Müller, Hermelijn H. Smits and Christian Taube

J Immunol published online 19 January 2018
<http://www.jimmunol.org/content/early/2018/01/19/jimmunol.1700987>

Supplementary Material <http://www.jimmunol.org/content/suppl/2018/01/19/jimmunol.1700987.DCSupplemental>

Why *The JI*?

- **Rapid Reviews! 30 days*** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Speedy Publication!** 4 weeks from acceptance to publication

**average*

Subscription Information about subscribing to *The Journal of Immunology* is online at: <http://jimmunol.org/subscription>

Permissions Submit copyright permission requests at: <http://www.aai.org/About/Publications/JI/copyright.html>

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at: <http://jimmunol.org/alerts>



Therapeutic Application of an Extract of *Helicobacter pylori* Ameliorates the Development of Allergic Airway Disease

Yolanda van Wijck,* Stan de Kleijn,* Gerrit John-Schuster,* Tinne C. J. Mertens,* Pieter S. Hiemstra,* Anne Müller,[†] Hermelijn H. Smits,[‡] and Christian Taube*,¹

Epidemiological and experimental studies have shown that exposure to the gastric bacterium *Helicobacter pylori*, especially in early life, prevents the development of asthma. Recent mouse studies have shown that this protective effect does not require live bacteria and that treatment with an extract of *H. pylori* in neonates prevents the development of airway inflammation and goblet cell metaplasia. In the current study, the effect of administration of an extract of *H. pylori* was assessed in a therapeutic study design with application of the extract just prior to allergen challenge. C57BL/6 mice were sensitized and challenged with OVA or house dust mite. Treatment with *H. pylori* extract just prior to the challenge significantly reduced airway inflammation, as assessed in bronchoalveolar lavage fluid and lung tissue, and reduced airway remodeling, as assessed by goblet cell quantification. These effects were apparent in the OVA model and in the house dust mite model. Injection of *H. pylori* extract reduced the processing of allergen by dendritic cells in the lungs and mediastinal lymph node. Bone marrow–derived dendritic cells exposed to *H. pylori* extract were affected with regard to their ability to process Ag. These data show that application of *H. pylori* extract after sensitization effectively inhibits allergic airway disease. *The Journal of Immunology*, 2018, 200: 000–000.

Asthma is one of the most prevalent chronic diseases worldwide (1), and a further increase in asthma prevalence is expected (2). Asthma is a heterogeneous syndrome that is characterized by airway obstruction, hyper-responsiveness, and inflammation (3). As part of the heterogeneity of this syndrome, patients can be assigned to certain phenotypes based on clinical or immunological criteria (4). One of the most prominent clinical phenotypes is allergic asthma, which is characterized by the induction of inflammation following exposure of sensitized patients to allergens. Indeed, the immunological pathophysiology underlying allergic asthma is well described and involves complex interactions of epithelial cells, dendritic cells (DCs), mast cells, T cells, B cells, and eosinophilic granulocytes (3, 5, 6). So far, only symptomatic treatment is available for these patients.

The increase in the prevalence of asthma has been linked to changes in environmental exposure, such as tobacco smoke and traffic emission, as well as to changes in microbial exposure (7, 8). High hygienic standards seem to predispose to the development of

allergies, such as allergic asthma (9), which was later postulated as the hygiene hypothesis. It is now argued that changes in exposure to microorganisms (10), as well as other factors associated with a Western lifestyle, such as diet, antibiotic use, and cesarean sections (11), are related to the altered composition of our microbiota, which has been implicated in the loss of tolerance to allergens. An example of a microorganism that is found less frequently in the human microbiota today is the pathobiont *H. pylori* (12). *H. pylori* is a Gram-negative spirochete that resides in the stomach (13) and is known for its association with gastritis, gastric ulcer, and gastric cancer (14). Interestingly, multiple epidemiological studies have reported a protective effect of infection with *H. pylori* on the development of asthma (15, 16).

In addition, an increasing number of experimental studies showed that, especially, neonatal infection with *H. pylori* or application of an extract of the bacteria from neonatal age on reduced allergic airway disease (17–19). Further mechanistic studies showed that protection induced by *H. pylori* involved induction of tolerogenic DCs and regulatory T cells (Tregs) via IL-18- and IL-10-dependent mechanisms. However, the protective effects of *H. pylori* have been exclusively assessed in prophylactic models, investigating animals infected or treated before sensitization.

Therefore, in this study, the therapeutic effect of an *H. pylori* extract was assessed in sensitized animals that were treated just prior to airway challenge. Treatment with *H. pylori* extract just prior to airway challenge was found to inhibit airway inflammation and remodeling, and changes in the DC compartment were observed. Importantly, this effect was observed in two models of allergic airway disease. These data show the therapeutic potential of *H. pylori* components for the treatment of allergic asthma.

Materials and Methods

Animals

Eight- to twelve-week-old female C57BL/6 mice were purchased from Charles River ('s-Hertogenbosch, the Netherlands). Animals were housed under specific pathogen-free conditions in individually ventilated cages, with free access to food and water, at the animal facilities of the Leiden University Medical Center. All experiments were approved by the Ethics

*Department of Pulmonology, Leiden University Medical Center, 2300 RC Leiden, the Netherlands; [†]Institute of Molecular Cancer Research, University of Zürich, CH-8057 Zürich, Switzerland; and [‡]Department of Parasitology, Leiden University Medical Center, 2300 RC Leiden, the Netherlands

¹Current address: Department of Pulmonary Medicine, University Hospital Essen, Ruhrlandklinik, West German Lung Center, Essen, Germany.

ORCIDs: 0000-0003-4384-736X (Y.v.W.); 0000-0002-0238-5982 (P.S.H.).

Received for publication July 10, 2017. Accepted for publication December 18, 2017.

This work was supported by the Longfonds (Consortium Grant 5115015).

Address correspondence and reprint requests to Yolanda van Wijck, Department of Pulmonology, Leiden University Medical Center, Albinusdreef 2, Internal postcode C-02-062, Leiden, the Netherlands. E-mail address: y.van_wijck@lumc.nl

The online version of this article contains supplemental material.

Abbreviations used in this article: AAI, allergic airway inflammation; alum, aluminum hydroxide; BALF, bronchoalveolar lavage fluid; BMDC, bone marrow-derived DC; DC, dendritic cell; HDM, house dust mite; i.n., intranasal(ly); MHCII, MHC class II; mLN, mediastinal lymph node; PAS, periodic acid–Schiff; Treg, regulatory T cell.

Copyright © 2018 by The American Association of Immunologists, Inc. 0022-1767/18/\$35.00

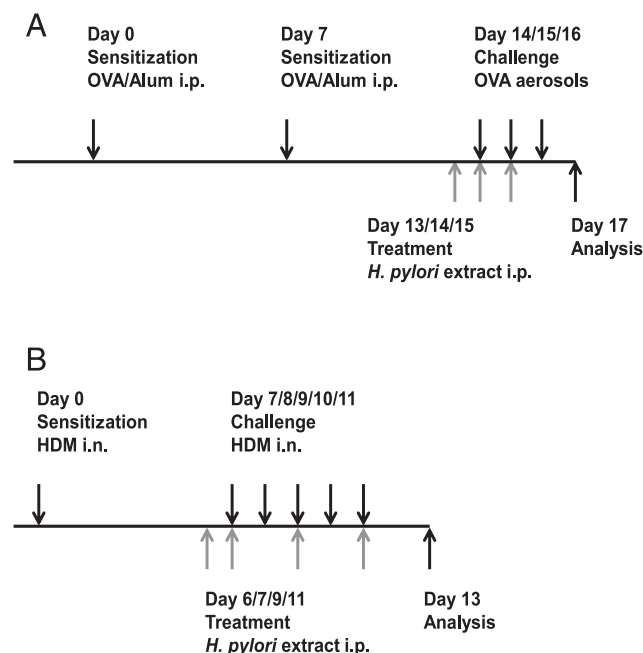


FIGURE 1. Experimental setup of models of AAI. **(A)** OVA/alum model. C57BL/6 mice were sensitized by i.p. injection of OVA/alum on days 0 and 7. Mice were treated i.p. with an extract of *H. pylori* on days 13–15 and challenged with OVA aerosols on days 14–16. Analyses were performed on day 17. **(B)** HDM model. C57BL/6 mice were sensitized by i.n. application of HDM. Mice were treated i.p. with an extract of *H. pylori* on days 6, 7, 9, and 11 and challenged i.n. with HDM on days 7–11. Analyses were performed on day 13.

Committee for Animal Experimentation of the University of Leiden (Dieren Experimentele Commissie 12246) and were conducted under strict governmental and international guidelines in accordance with EU Directive 2010/63/EU.

Experimental protocols for allergic airway inflammation and intervention

An allergic reaction to OVA in the lungs of mice was induced by i.p. injection with 10 μ g of OVA (InvivoGen, San Diego, CA) suspended in 1 mg of aluminum hydroxide (alum) (Imject alum adjuvant; Pierce, Thermo Fisher Scientific, Waltham, MA) in 500 μ l of PBS on days 0 and 7, followed by three aerosol challenges with 1% (w/v) OVA (albumin, from chicken egg white, grade III; Sigma-Aldrich, Zwijndrecht, the Netherlands) in PBS using an ultrasonic nebulizer (NE-U17; Omron, Osaka, Japan) for 25 min on days 14, 15, and 16 (Fig. 1A). Control mice were injected i.p. with PBS and challenged with 1% OVA in PBS. Mice were treated i.p. with 200 μ g of *H. pylori* extract in 100 μ l of PBS or 100 μ l of PBS as a control on days 13, 14, and 15. When mice were treated and challenged on the same day, they were challenged 1 h after the treatment. Mice were analyzed 1 d after the last challenge. House dust mite (HDM) allergic airway inflammation (AAI) was induced by one intranasal (i.n.) sensitization with 1 μ g of HDM (Greer, Lenoir, NC) in 50 μ l of PBS on day 0, followed by five i.n. challenges with 10 μ g of HDM in 50 μ l of PBS on days 7, 8, 9, 10, and 11 (Fig. 1B). Intranasal administration was performed under isoflurane anesthesia (3%, 0.8 l/min). Control mice were sensitized with 1 μ g of HDM and challenged with PBS. On days 6, 7, 9, and 11, *H. pylori* extract (200 μ g) in 100 μ l of PBS or 100 μ l of PBS as a control was applied i.p. These mice were analyzed on day 13. After euthanasia with an overdose of sodium pentobarbital (Euthasol; AST Farma, Oudewater, the Netherlands), blood was collected from the vena cava, and the lungs of mice were lavaged three times with 1 ml of PBS via a tracheal cannula and then were perfused with PBS via the heart. The left lobe of the lungs was collected for flow cytometry, whereas the right lobe was inflated with 3.9% formaldehyde (Sigma-Aldrich) in PBS to study the histology.

Preparation of *H. pylori* extract

Oxidase, catalase, and urease production by *H. pylori* premouse SS1 strain grown on *H. pylori* culture plates (bioMérieux, Benelux B.V., Zaltbommel,

the Netherlands) was confirmed before bacteria were transferred to *Bruce* broth supplemented with 10% FBS (Life Technologies, Thermo Fisher Scientific). Bacteria were grown under microaerophilic conditions (6% O₂, 7.1% CO₂, 7.1% H₂, and 79.7% N₂) (Anoxomat; Mart Microbiology, Drachten, the Netherlands) in a shaking incubator (New Brunswick Scientific Europe B.V., Nijmegen, the Netherlands) at 185 rpm and 37°C overnight. The following day, the culture was expanded twice by adding two parts medium to one part culture. After overnight culturing, the bacteria were harvested by centrifugation. Gram staining was performed to check the culture. Pellets of multiple cultures were pooled, washed in PBS, and freeze-thawed in nitrogen. The bacteria were lysed using a French pressure cell press (Cell Pressure Homogenizer; Stansted Fluid Power, Harlow, U.K.) at 3000 bar. The product was centrifuged, and the supernatant was filtered through a 0.2- μ m filter. Protein content was determined by bicinchoninic acid (Pierce BCA Protein Assay Kit; Thermo Fisher Scientific) and adjusted to the desired concentration using PBS. Approximately 30 mg of extract was obtained from 8 l of bacterial culture. Toxicity of the extract was ruled out by testing in various in vitro assays (unpublished observations, Y. van Wijck).

Anti-OVA/HDM IgE and IgG1

OVA/HDM-specific IgE and IgG1 Abs in the serum were measured by ELISA. Nunc MaxiSorp plates (Sigma-Aldrich) were coated with 25 μ g/ml OVA (grade III; Sigma-Aldrich) or 50 μ g/ml HDM in 0.1 M sodium carbonate buffer (pH 9.6). Nonspecific binding plates were blocked with 1% (w/v) BSA (Sigma-Aldrich) in PBS. Samples and an anti-OVA IgE standard (Bio-Rad, Veenendaal, the Netherlands) were incubated, and OVA/HDM-specific Abs were detected by biotin-labeled anti-mouse IgE (clone 23G3; SouthernBiotech, Birmingham, AL) and biotin-labeled anti-mouse IgG1 (clone A85-1; BD Pharmingen, Becton Dickinson B.V., Breda, the Netherlands), followed by streptavidin coupled to HRP (Sanquin, Amsterdam, the Netherlands) and a 3,3',5,5'-tetramethylbenzidine reaction. Plates were measured on a Bio-Rad iMark microplate reader at 450 nm. For anti-OVA IgE, the detection limits were determined by the upper and lower concentration of the linear part of the standard curve. Values higher or lower than these values were set to the detection limits.

Flow cytometry

Cells in bronchoalveolar lavage fluid (BALF) were counted using Türk's solution (Merck, Schiphol-Rijk, the Netherlands). A single-cell suspension of the lung tissue cells was obtained by dissecting the tissue into small pieces, followed by Collagenase/DNase (Collagenase type I of *Clostridium histolyticum* [Calbiochem, San Diego, CA]; DNase I [Sigma-Aldrich]) treatment for 45 min, dispersion through a 70- μ m cell strainer (BD Falcon, Becton Dickinson B.V.), and lysis of the RBCs. Lung tissue cells were counted on a Countess automatic cell counter (Invitrogen, Thermo Fisher Scientific). Next, cells in BALF and lung tissue were washed in PBS, stained with LIVE/DEAD aqua stain (Invitrogen, Thermo Fisher Scientific), washed, and fixed with 2% formaldehyde (Merck) in PBS. The following Abs were used to distinguish cell populations in BALF by flow cytometry: Ly-6G and Ly-6C-FITC (Gr-1) (RB6-8C5; BD Biosciences, Becton Dickinson B.V.), Siglec-F-PE (E50-2440; BD Biosciences, Becton Dickinson B.V.), CD3-PerCP-eFluor 710 (17A2; eBioscience, Thermo Fisher Scientific), CD11b-PE-Cy7 (M1/70; eBioscience, Thermo Fisher Scientific), CD45R-allophycocyanin-eFluor 780 (B220) (RA3-6B2;

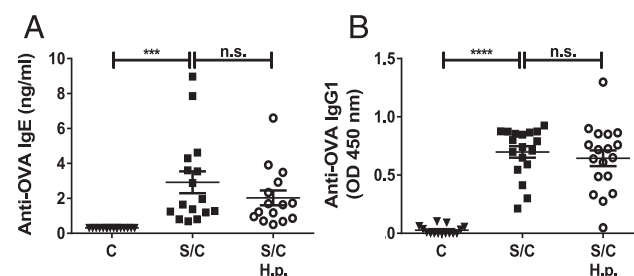


FIGURE 2. OVA-specific Abs in serum. **(A)** Anti-OVA IgE, measured at 450 nm and converted to ng/ml. **(B)** Anti-OVA IgG1 (shown as OD₄₅₀ values). Results are presented as mean $\pm$ SEM of three independent experiments, $n = 15$ –18 per group. *** $p \leq 0.001$, **** $p \leq 0.0001$, ANOVA with the Dunnett multiple-comparison correction. C, challenged; n.s., nonsignificant; S/C, sensitized and challenged; S/C H.p., sensitized, treated with *H. pylori* extract, and challenged.

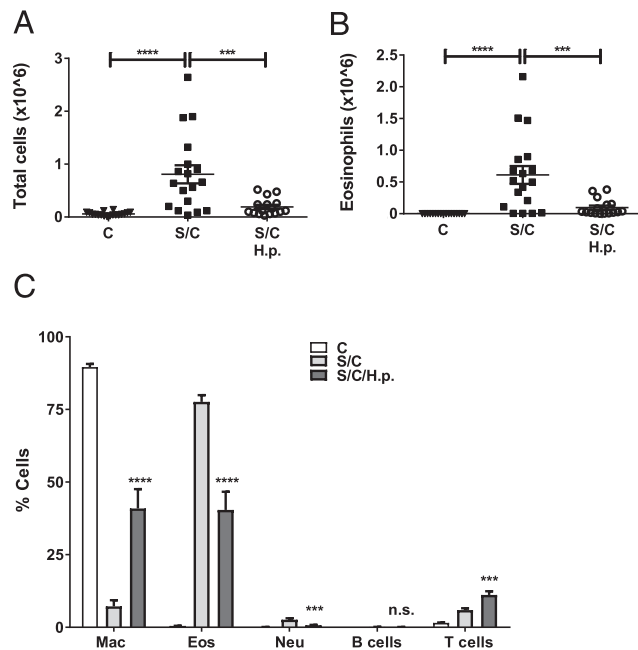


FIGURE 3. Characterization of cells present in BALF after treatment of OVA/alum-induced AAI with an extract of *H. pylori*. (A) Total number of cells present in BALF, as determined by counting in Türk's solution. (B) Exact number of eosinophilic granulocytes present in BALF, as determined by multiplying the total number of cells in BALF by the percentage of eosinophils determined by flow cytometry. (C) Flow cytometric analysis of cells present in BALF. Results are presented as mean $\pm$ SEM of three experiments, $n = 14$ – 18 per group. *** $p \leq 0.001$, **** $p \leq 0.0001$, ANOVA with the Dunnett multiple-comparison correction. C, challenged; Eos, eosinophils; Mac, alveolar macrophages; Neu, neutrophils; n.s., nonsignificant; S/C, sensitized and challenged; S/C H.p., sensitized, treated with *H. pylori* extract, and challenged.

eBioscience, Thermo Fisher Scientific), and CD11c-V450 (HL3; BD Biosciences, Becton Dickinson B.V.). To analyze DC populations in the lungs, the following Abs were used: CD3-FITC (17A2; eBioscience, Thermo Fisher Scientific), CD19-FITC (MB19-1; eBioscience, Thermo Fisher Scientific), NK1.1-FITC (PK136; eBioscience, Thermo Fisher Scientific) and Ly-6G and Ly-6C-FITC (Gr-1) (RB6-8C5; BD Biosciences, Becton Dickinson B.V.) in a dump channel, Siglec-F-PE (E50-2440; BD Biosciences, Becton Dickinson B.V.), CD64-PerCP/Cy5.5 (FcγRI) (X54-5/7.1; BioLegend, San Diego, CA), CD11b-PE-Cy7 (M1/70; eBioscience, Thermo Fisher Scientific), CD103-Alexa Fluor 647 (2E7; BioLegend), MHC class II (MHCII) (I-A/I-E)-allophycocyanin-eFluor 780 (M5/114.15.2; eBioscience, Thermo Fisher Scientific), and CD11c-V450 (HL3; BD Biosciences, Becton Dickinson B.V.). Ab mixes were supplemented with mouse FcγRII/III-binding inhibitor (2.4G2; Bioceros, Utrecht, the Netherlands). Cells were measured on a FACSCanto II (BD Biosciences, Becton Dickinson B.V.), and analysis was performed using FlowJo (v7.6.5) software (TreeStar, Ashland, OR). Gating strategies can be found in Supplemental Figs. 1 and 2. Gates were placed according to unstained and Fluorescence Minus One samples.

Histology

The formaldehyde-inflated tissue was fixed for 48 h at room temperature. Four-micrometer paraffin sections were stained with H&E (Klinipath, Duiven, the Netherlands). Peribronchial inflammation, as examined by H&E staining, was scored on a scale from 0 to 4 by two investigators who were blinded to the identity of the slides. Alcian blue–periodic acid–Schiff (PAS) (both from Sigma-Aldrich) staining was performed to quantify the extent of goblet cell metaplasia. For analysis, digital images of Alcian blue–PAS–stained lung sections were taken at 100 $\times$ magnification on an Olympus BX41 microscope (Olympus Nederland B.V., Leiderdorp, the Netherlands) and analyzed with ImageJ software. Briefly, epithelial regions were selected and separated into red, green, and blue channels. The color threshold was manually set in green channelled images in such a way that the magenta mucin content matched the color threshold by comparing the original

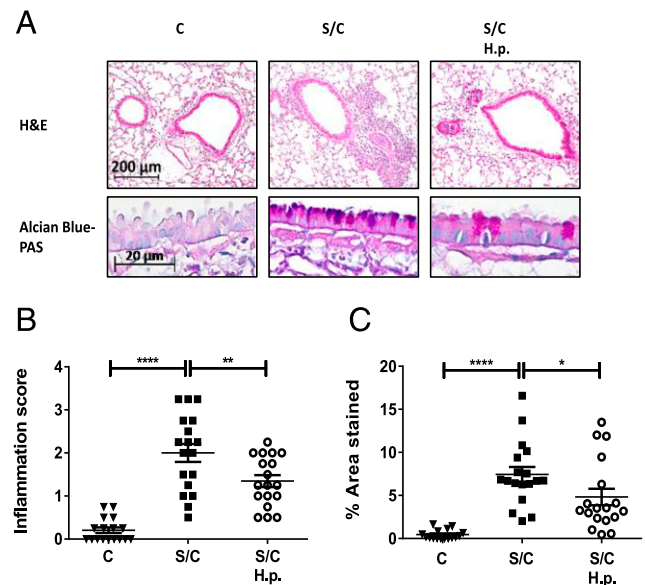


FIGURE 4. Lung tissue histology after *H. pylori* extract treatment of OVA/alum-induced AAI. (A) Histological analysis of peribronchial and perivascular inflammation by H&E staining of lung tissue slides (original magnification $\times 200$) and goblet cell metaplasia by Alcian blue–PAS staining (original magnification $\times 400$). (B) Inflammation score based on H&E staining. (C) Quantification of mucus-producing cells, as assessed by Alcian blue–PAS staining. Results are presented as mean $\pm$ SEM of three experiments, $n = 17$ – 18 per group. * $p \leq 0.05$, ** $p \leq 0.01$, **** $p \leq 0.0001$, ANOVA with the Dunnett multiple-comparison correction. C, challenged; S/C, sensitized and challenged; S/C H.p., sensitized, treated with *H. pylori* extract, and challenged.

Alcian blue–PAS–stained images simultaneously. These images were converted into binary images, and the area fraction of mucin density (%) was calculated using “summarize” and “analyze particle” options.

Assessment of processing of allergen by DCs

Mice were sensitized to OVA by two i.p. injections with 10 μ g of OVA (InvivoGen) suspended in 1 mg of alum (Pierce, Thermo Fisher Scientific) in 500 μ l of PBS on days 0 and 7. To analyze the effect of the extract on processing of allergen by DCs, mice were treated i.p. with 200 μ g of extract in 100 μ l of PBS on days 13 and 14. An hour after the second treatment, mice were challenged i.n. with DQ OVA (40 μ g in 50 μ l of PBS) (Thermo Fisher Scientific), a self-quenched form of OVA that emits light only when it is processed by proteases. Processing was analyzed 24 h after the challenge. Single-cell suspensions of the lung and mediastinal lymph node (mLN) were stained with Aqua and fixed in formaldehyde. Subsequently, cells were stained for CD19–allophycocyanin–Cy7 (1D3; BD Pharmingen, Becton Dickinson B.V.), CD11c-V450 (HL3; BD Biosciences, Becton Dickinson B.V.), and MHCII (I-A/I-E)-allophycocyanin (M5/114.15.2; eBioscience, Thermo Fisher Scientific) in the presence of mouse FcγRII/III-binding inhibitor (2.4G2; Bioceros) and analyzed by flow cytometry.

Bone marrow–derived DC isolation and processing of Ag

Bone marrow was isolated from C57BL/6 mice. Cells were cultured in RPMI 1640 GlutaMAX (Thermo Fisher Scientific) supplemented with 100 U/ml penicillin (Sigma-Aldrich), 100 μ g/ml streptomycin (Sigma-Aldrich), 5×10^{-5} M 2-ME (Sigma-Aldrich), 5% heat-inactivated FBS (Bodinco, Alkmaar, the Netherlands), and 20 ng/ml GM-CSF (PeproTech, London, U.K.). The medium was refreshed on days 3 and 6. On day 7, cells were replated, rested for 3 h, and stimulated with 20 μ g/ml nonlabeled OVA (InvivoGen) or 1 μ g/ml DQ OVA (Thermo Fisher Scientific) in the presence or absence of 100 μ g/ml *H. pylori* extract for 24 h. Cells were stained with Aqua, labeled with CD11c-V450 (HL3; BD Biosciences, Becton Dickinson B.V.) and MHCII (I-A/I-E)-allophycocyanin (M5/114.15.2; eBioscience, Thermo Fisher Scientific) in the presence of mouse FcγRII/III-binding inhibitor (2.4G2; Bioceros), and analyzed by flow cytometry.

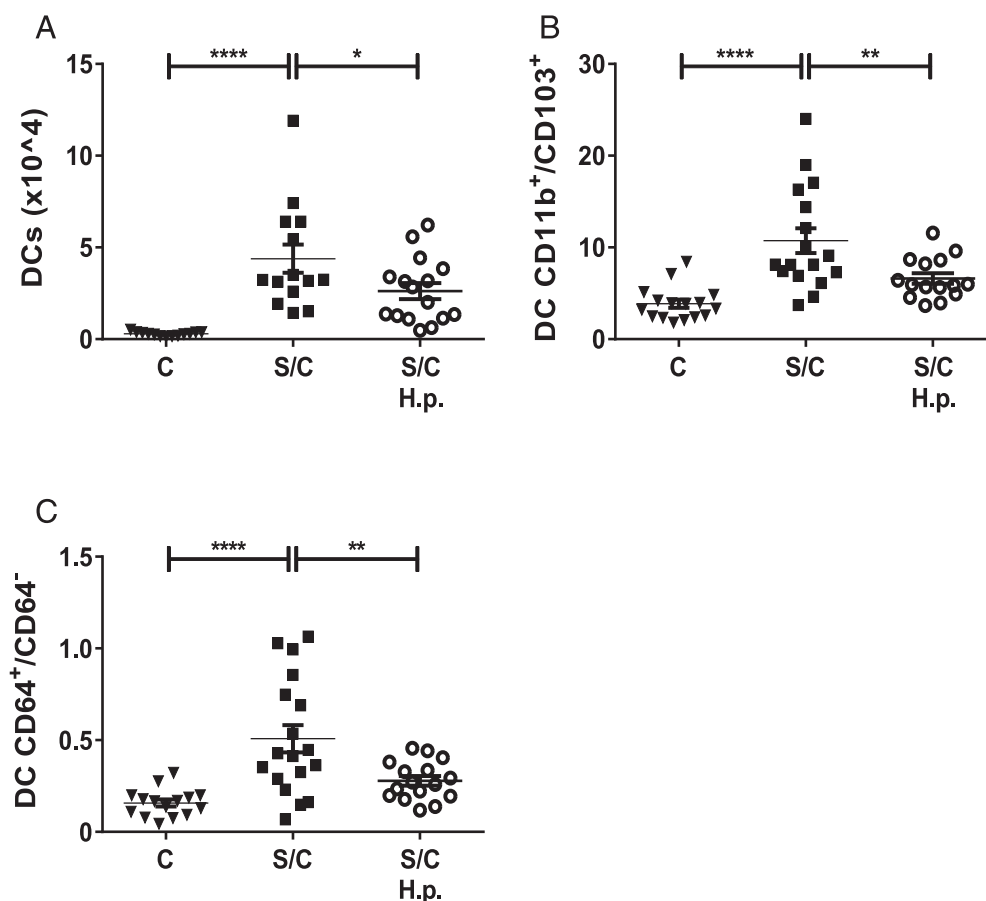


FIGURE 5. Characterization of DC subsets in lung tissue after *H. pylori* extract treatment of OVA/alum-induced AAI. **(A)** Exact number of DCs present in the lung tissue, quantified by multiplying the total cell count by the percentage of DCs assessed by flow cytometry. **(B)** Flow cytometric analysis of the ratio of CD11c⁺MHCII⁺CD11b⁺ (CD64⁻ and CD64⁺) DCs/CD11c⁺MHCII⁺CD103⁺ DCs in lung tissue. **(C)** Flow cytometric analysis of the ratio of CD11c⁺MHCII⁺CD11b⁺CD64⁺ DCs/CD11c⁺MHCII⁺CD11b⁺CD64⁻ DCs in lung tissue. Results are presented as mean ± SEM of three experiments, $n = 14$ –18 per group. * $p \leq 0.05$, ** $p \leq 0.01$, **** $p \leq 0.0001$, ANOVA with the Dunnett multiple-comparison correction. C, challenged; S/C, sensitized and challenged; S/C H.p., sensitized, treated with *H. pylori* extract, and challenged.

Statistical analysis

Graphs were designed and statistical analysis was performed in GraphPad Prism 7.02 (GraphPad Software, La Jolla, CA). Data are shown as means ± SEM. Differences were considered significantly different at p values < 0.05, as determined by ANOVA and the Dunnett multiple-comparison analysis.

Results

Application of an extract of *H. pylori* to sensitized animals ameliorates allergic airway disease following allergen challenge

The development of AAI was assessed in OVA-sensitized mice, which received *H. pylori* extract by i.p. injection and then were challenged with OVA aerosols (experimental setup in Fig. 1A). Sensitization was confirmed by increased serum levels of allergen-specific IgE and IgG1 (Fig. 2). Sensitized animals treated with *H. pylori* extract exhibited levels of allergen-specific IgE and IgG1 comparable to the sensitized and challenged controls. Sensitized and challenged animals showed increased numbers of total cells and eosinophils in flow cytometry analysis of BALF (gating Supplemental Fig. 1) compared with animals that were challenged but not sensitized (Fig. 3A, 3B). Treatment with *H. pylori* extract significantly decreased the total cell count, eosinophil count, and percentage of eosinophils in BALF (Fig. 3). This anti-inflammatory effect was also apparent when analyzing lung tissue. Indeed, sensitized and challenged animals showed increased airway inflammation (Fig. 4A, 4B), as

well as goblet cell metaplasia (Fig. 4A, 4C), compared with animals that were only challenged. Peribronchial and perivascular inflammation in allergic mice was significantly reduced upon treatment with *H. pylori* extract. Furthermore, mucus-producing cells in the airways were decreased in mice treated with *H. pylori* extract.

DCs play a pivotal role in the development and maintenance of allergic airway disease. DC subtypes were assessed in the lung by flow cytometry based on previously published methods (Supplemental Fig. 2). As expected, sensitization and challenge resulted in an increase in the number of DCs (CD11c⁺MHCII⁺) in the lung (Fig. 5A). Interestingly, the overall number of DCs was decreased in animals treated with *H. pylori* extract. In addition, treatment with *H. pylori* extract altered the composition of DC subtypes in the lung. The ratio of DCs with a proinflammatory phenotype to DCs with an anti-inflammatory phenotype was reduced after treatment with the extract (Fig. 5B). Animals treated with *H. pylori* extract showed a reduction in the ratio of proinflammatory monocyte-derived CD11b⁺CD64⁺ DCs to conventional CD11b⁺CD64⁻ DCs (Fig. 5C).

Application of an extract of *H. pylori* to sensitized animals ameliorates HDM-induced allergic airway disease following allergen challenge

To further examine the effect of an extract of *H. pylori* on allergic airway disease, a model with a human relevant allergen and a

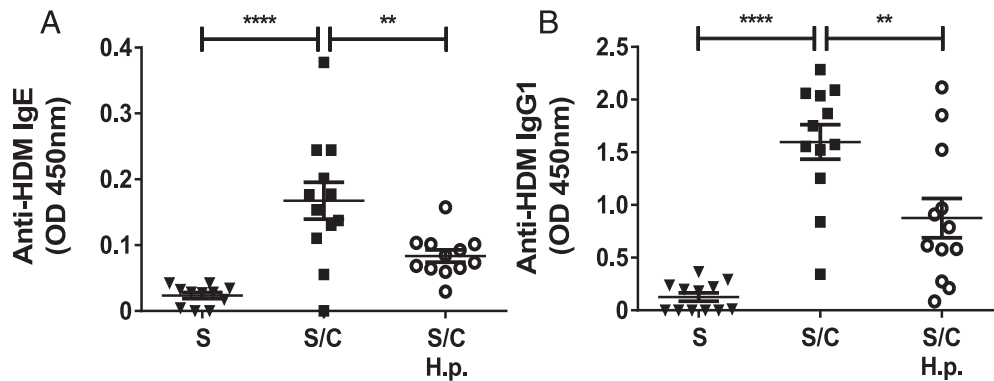


FIGURE 6. HDM-specific Abs in serum. **(A)** Anti-HDM IgE. **(B)** Anti-HDM IgG1. Results are presented as mean $\pm$ SEM of two experiments, $n = 11$ – 12 per group. $**p \leq 0.01$, $****p \leq 0.0001$, ANOVA with the Dunnett multiple-comparison correction. S, sensitized; S/C, sensitized and challenged; S/C H.p., sensitized, treated with *H. pylori* extract, and challenged.

route of allergen exposure that is more comparable to human exposure was used. Mice were sensitized to HDM by i.n. application and received *H. pylori* extract by i.p. injections prior to and during the allergen challenges (Fig. 1B). In contrast to the findings in the OVA/alum model, allergen-specific IgE and IgG1 serum levels were significantly reduced following treatment with the extract in this model (Fig. 6). A trend toward a lower total cell count in BALF was observed after treatment (Fig. 7A). Flow cytometry analysis showed a significant reduction in the absolute number and percentage of eosinophils in BALF following treatment with *H. pylori* extract (Fig. 7B, 7C). In addition, allergen-induced lung tissue inflammation and mucus-producing cells in the airways were significantly reduced upon treatment with *H. pylori* extract (Fig. 8). Interestingly, treatment with *H. pylori* extract resulted in a trend toward decreased DCs in the lung tissue

(Fig. 9A) and a shift in DC phenotype, with a significant effect on the ratio of CD11b⁺/CD103⁺ DCs and a trend toward a reduced ratio of CD11b⁺CD64⁺/CD11b⁺CD64[−] DCs (Fig. 9B, 9C).

Treatment with *H. pylori* extract reduces Ag processing by DCs

To further assess whether the observed changes in the DC compartment following treatment with *H. pylori* extract affected DC function, we analyzed Ag processing by DCs. Mice sensitized to OVA were treated with *H. pylori* extract and subsequently challenged once with DQ OVA by i.n. application. Detection of DQ OVA by FACS is only possible after Ag processing, because proteases cleave the quenching molecules from the DQ OVA, resulting in fluorescence. Treatment with *H. pylori* extract significantly reduced the number of fluorescent DCs in the lung (Fig. 10A, 10B). In the mLN, a trend toward a decrease in fluorescent DCs was

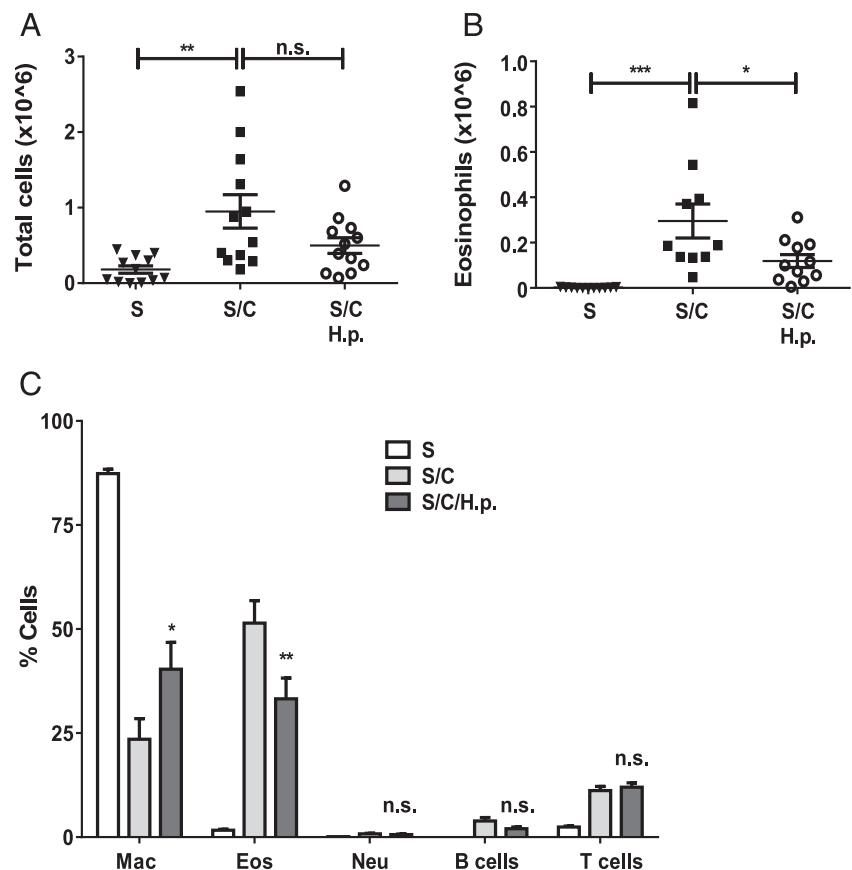
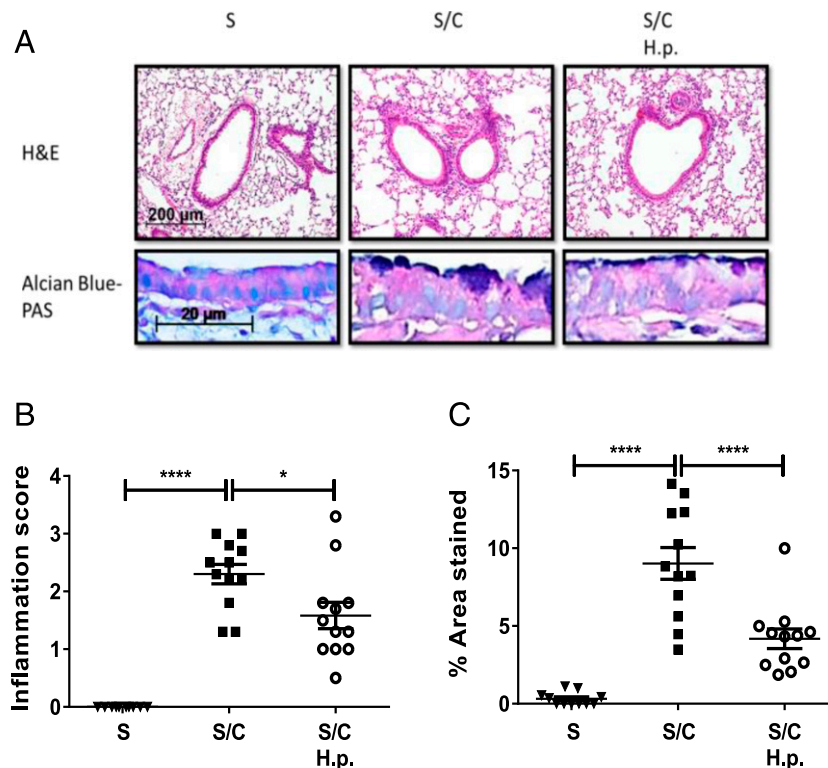


FIGURE 7. Characterization of cells present in BALF after treatment of HDM-induced AAI with an extract of *H. pylori*. **(A)** Total number of cells present in BALF, as determined by counting in Türk's solution. **(B)** Exact amount of eosinophilic granulocytes present in BALF, as determined by multiplying the total amount of cells in BALF by the percentage of eosinophils determined by flow cytometry. **(C)** Flow cytometric analysis of cells present in BALF. Results are presented as mean $\pm$ SEM of two experiments, $n = 9$ – 12 per group. $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$, ANOVA with the Dunnett multiple-comparison correction. Eos, eosinophils; Mac, alveolar macrophages; Neu, neutrophils; n.s., nonsignificant; S, sensitized; S/C, sensitized and challenged; S/C H.p., sensitized, treated with *H. pylori* extract, and challenged.

FIGURE 8. Lung tissue histology after *H. pylori* extract treatment of HDM-induced AAI. **(A)** Histological analysis of peribronchial and perivascular inflammation by H&E staining of lung tissue slides (original magnification $\times 200$) and goblet cell metaplasia by Alcian blue–PAS staining (original magnification $\times 400$). **(B)** Inflammation score based on H&E staining. **(C)** Quantification of mucus-producing cells assessed by Alcian blue–PAS staining. Results are presented as mean $\pm$ SEM of two experiments, $n = 10$ – 12 per group. $^*p \leq 0.05$, $^{****}p \leq 0.0001$, ANOVA with the Dunnett multiple-comparison correction. n.s., nonsignificant; S, sensitized; S/C, sensitized and challenged; S/C H.p., sensitized, treated with *H. pylori* extract, and challenged.



observed (Fig. 10C). To analyze whether the effect of the extract on processing by DCs could be due to a direct effect on DCs, bone marrow–derived DCs (BMDCs) were isolated and exposed to DQ OVA in the presence or absence of *H. pylori* extract for 24 h. In this in vitro culture, exposure of DCs to *H. pylori* extract resulted in a significant reduction in fluorescence compared with DCs that were only exposed to DQ OVA (Fig. 10D, 10E).

Discussion

Previous studies have shown that infection with *H. pylori*, as well as exposure to an extract of *H. pylori* from especially neonatal age on, prior to sensitization, reduces the development of allergic airway disease (17–19). The aim of this study was to further examine whether administration of *H. pylori* extract after sensitization, and only prior to airway challenge, was also effective in reducing the hallmarks of allergic airway disease, thereby modeling a more therapeutic application. Sensitization leads to an increase in Ag-specific allergen capture and processing by various APCs in the airway mucosa at challenge, facilitated by allergen-specific Abs (21). Furthermore, there is increased DC precursor recruitment but also DC proliferation (22) and rapid T cell activation at challenge (21). Therefore, therapeutic interventions to reduce AAI at this stage are more challenging compared with preventive strategies, and many compounds that have been effective in prophylactic approaches failed to reduce allergic airway disease when used in therapeutic models. This study shows that systemic treatment of sensitized mice with an extract of *H. pylori* before allergen exposure decreases the development of AAI. This was observed in a model of systemic sensitization following i.p. injection of the allergen OVA, as well as in a model of sensitization following HDM allergen administration into the lung. The OVA/alum model is a well-known model that has contributed to the understanding of the mechanisms of AAI (23). In contrast, HDM is a human-relevant allergen that is applied i.n. without an adjuvant for sensitization or challenge, which is a more common

route than the i.p. injection used for sensitization in the OVA/alum model (24, 25).

It is now well established that exposure to microorganisms is an important factor in shaping the immune system and also is important for the susceptibility to develop allergies and asthma (26). This holds true for the exposure to exogenous bacteria, and different research groups have shown that exposure to bacteria prior to or starting at the time of sensitization reduces the development of allergic disease in various models (27–30). There is also increasing evidence that microbiota contribute to the susceptibility to develop an allergic disease, and changes in the composition of the microbiota have been linked to the increased prevalence of allergies and asthma (31, 32). Indeed, it has been demonstrated that the composition and diversity of gut and lung microbiota influence the susceptibility for the development of allergic airway disease (33, 34). As one specific component of the human microbiome, *H. pylori* (the dominant bacterial species in the gastric mucosa) has received increasing attention with regard to its inverse association with asthma (15). Several previous studies have demonstrated that infection with (but also an extract of) *H. pylori* has a protective effect on the development of allergen-induced airway disease (17–19). The present study adds novel insights, showing the efficacy of the therapeutic use of an extract of *H. pylori*. Systemic application of *H. pylori* extract significantly reduced AAI, as well as goblet cell metaplasia, and these effects were apparent in two models of allergic airway disease. This is of interest, because these findings suggest a potential therapeutic effectiveness of this extract.

Gnotobiotic mice would make it possible to analyze the effects of *H. pylori* on AAI in the absence of other microorganisms. However, this makes it harder to translate the effects to humans, which have a large microbiome, and it is not clear whether *H. pylori* affects the microbiome (35, 36). In this study, an extract of the bacteria is used, and the treatment is only for a few days; therefore, it is unlikely that the extract affects AAI by affecting the microbiome. The protective effect of infection or *H. pylori* extract

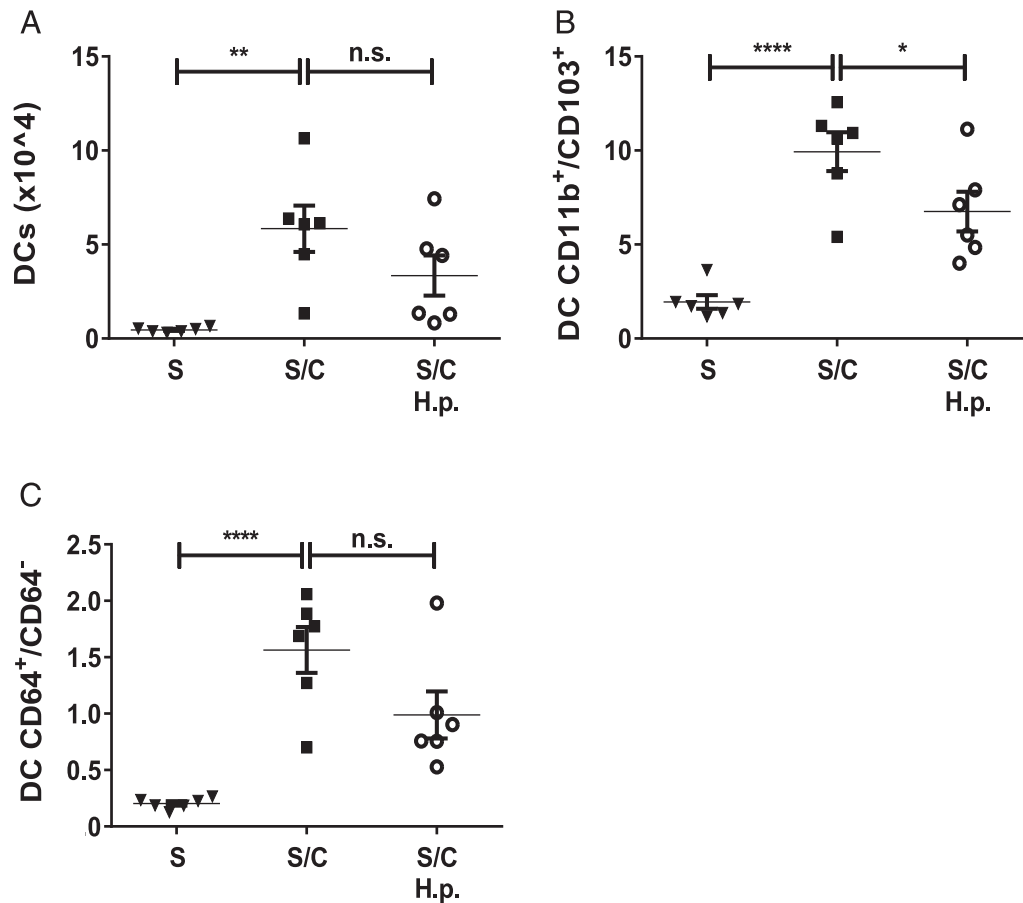


FIGURE 9. Characterization of DC subsets in lung tissue after *H. pylori* extract treatment of HDM-induced AAI. **(A)** Exact number of DCs present in the lung tissue, quantified by multiplying the total cell count by the percentage of DCs assessed by flow cytometry. **(B)** Flow cytometric analysis of the ratio of CD11c⁺MHCII⁺CD11b⁺ (CD64⁻ and CD64⁺) DCs/CD11c⁺MHCII⁺CD103⁺ DCs in lung tissue. **(C)** Flow cytometric analysis of the ratio of CD11c⁺MHCII⁺CD11b⁺CD64⁺ DCs/CD11c⁺MHCII⁺CD11b⁺CD64⁻ DCs in lung tissue. Results are presented as mean $\pm$ SEM of one experiment, $n = 6$ per group. * $p \leq 0.05$, ** $p \leq 0.01$, **** $p \leq 0.0001$, ANOVA with the Dunnett multiple-comparison correction. n.s., nonsignificant; S, sensitized; S/C, sensitized and challenged; S/C H.p., sensitized, treated with *H. pylori* extract, and challenged.

for the development of allergic airway disease has been linked to tolerogenic reprogramming of DCs by the bacteria (18, 19). Therefore, we also analyzed the composition of DC subsets in this study. Interestingly, application of *H. pylori* extract resulted in a reduction in CD11b⁺CD64⁺ proinflammatory monocyte-derived DCs. This subset was not analyzed in the preventative setting. It is likely that the reduction in proinflammatory DCs is associated with an increase in tolerogenic DCs. Hoffmann et al. (37) described CD103⁺ DCs and plasmacytoid DCs as pulmonary DCs that can induce tolerance in allergic asthma. In the preventative setting, BATF3-dependent CD103⁺ DCs were indispensable for the protective effect of *H. pylori* infection or extract treatment on allergic airway disease (19). A decrease in the ratio of CD11b⁺/CD103⁺ DCs was also observed in our more therapeutic setting. Previous studies in animals showed that plasmacytoid DCs were also actively attracted to the lungs of allergic mice after *H. pylori* infection or extract treatment (19), but we did not analyze that subset in our more therapeutic experiments. Based on these data, it was hypothesized that inhibition of processing of Ag by DCs might be a mechanism by which treatment with an extract of *H. pylori* leads to reduced AAI. Indeed, our present in vitro experiments show that treatment with *H. pylori* extract reduced the Ag-processing capacity of BMDCs. This was detectable in vitro, as well as in vivo, because a reduced capacity of DCs in the lung (and to a lesser extent also in the mLN) to process allergen was observed in mice treated with *H. pylori* extract. Not only was the

absolute number of DCs that processed Ag reduced (which could also be explained by the overall reduction in DCs in the lungs and mLN), there was a trend toward a reduction in the fraction of DCs that processed Ag and in the amount of Ag processed by the DCs in mice treated with the extract (unpublished observations, Y. van Wijck). It is tempting to speculate that *H. pylori* might reduce Ag processing by DCs as a way to promote bacterial persistence in the host. It is unclear which components of the extract affect the DCs and by which mechanisms, as well as whether the same effect on Ag processing can be seen in the preventative setting. It would be interesting to analyze whether DC function is affected by vacuolating cytotoxin A, γ -glutamyl transpeptidase, and urease, which were shown to be of importance in the preventative setting (19, 38, 39). Similar to the effects of a helminth cathelicidin-like protein on macrophages (40), inhibition of endolysosomal acidification might be a mechanism by which DC processing is reduced by *H. pylori* extract. Furthermore, previous reports show reduced uptake of particles by monocyte-derived DCs when preincubated with *H. pylori* (41) and inhibition of Ag presentation by these DCs after *H. pylori* incubation (42). However, in the current study, reduced uptake of Ag, as assessed by adding fluorescently labeled Ag to BMDCs treated with the extract, was not detected (unpublished observations, Y. van Wijck), which reduces the likelihood that the effects on processing are caused by reduced uptake.

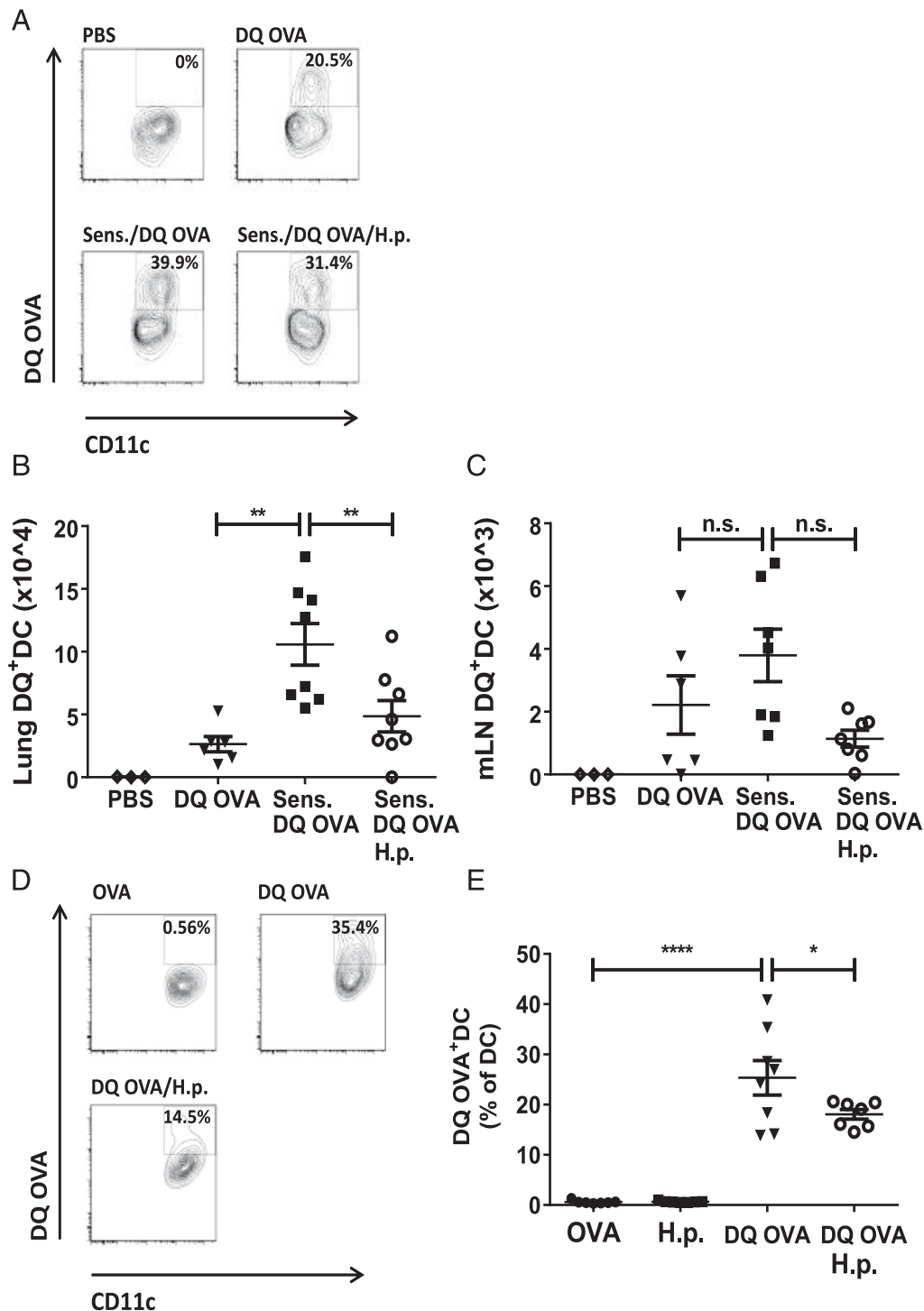


FIGURE 10. Processing of Ag by DCs after *H. pylori* extract treatment. **(A)** OVA-sensitized mice were treated with *H. pylori* extract on days 13 and 14. On day 14, they received 40 μ g of DQ OVA i.n. Lung tissue and mLN were collected 24 h later. Aqua-stained and formaldehyde-fixed cells were stained within a week. Single, live nonalveolar macrophages (Aqua⁺), CD19⁺CD11c⁺MHCII⁺ cells were gated to analyze the frequency of DCs that processed Ag. An example of the staining of lung tissue DCs is shown. **(B)** Quantification of DCs that processed Ag in the lung. **(C)** Quantification of DCs that processed Ag in the mLN. **(D)** BMDCs were stimulated with 1 μ g/ml OVA DQ in the presence or absence of *H. pylori* extract for 24 h. CD11c⁺MHCII⁺ live (Aqua⁺) single cells were gated to analyze the frequency of DCs that processed Ag. **(E)** Quantification of the frequency of BMDCs that processed Ag. Results are presented as mean $\pm$ SEM of two experiments, $n = 3-9$ per group (B and C) or $n = 7-8$ per group (E). * $p \leq 0.05$, ** $p \leq 0.01$, **** $p \leq 0.0001$, ANOVA with the Dunnett multiple-comparison correction. H.p., *H. pylori* extract; n.s., nonsignificant; Sens., sensitized.

Interestingly, the suppression of allergic airway disease was achieved by systemic application of the *H. pylori* extract, clearly showing a systemic effect of this therapy. It can be speculated that exposure to this bacterial extract shapes the immune system to a more tolerogenic response. However, this effect seems to be

independent of the mere exposure to LPS, because previous studies have shown that *Escherichia coli* or *Salmonella* extract was not effective in reducing the development of allergic airway disease (19). Following i.p. application, *H. pylori* extract is exposed to cells in the peritoneal cavity, which have been shown to

travel to the mLN and, therefore, could exert a suppressive function on the lungs. Indeed, Kool et al. (43) have shown that the mLN is involved in the reaction to an Ag following i.p. injection, even before the spleen and non-draining lymph nodes were fully involved. Therefore, i.p. injection of the extract seems to be a suitable route for inhibiting immune responses in the lungs. This was the case when sensitization was induced by i.p. injection of OVA, as well as in the HDM model, in which sensitization was induced by application of the allergen into the lung.

Lung function tests are often performed in clinical trials to assess the effectiveness of asthma medication. These tests should be evaluated in future studies. It would also be interesting to test the extract in a two-phase challenge model of AAI, in which sensitized and challenged mice are treated before a second challenge. Such repeated exposure models are more comparable to human disease, as patients with asthma are also repeatedly exposed to inhaled allergens.

In summary, an extract of *H. pylori* is effective in reducing the development of AAI in sensitized mice when used in a therapeutic fashion. A reduction in inflammation in BALF and tissue was observed in the OVA/alum and HDM models. The current data suggest that systemic application of *H. pylori* extract leads to induction of DCs with a less inflammatory phenotype and reduced Ag-processing ability. Therefore, the previously published prophylactic application can now be extended to prophylactic and therapeutic applications. Future studies are needed to assess whether this extract might be used in patients who are sensitized to allergens to prevent symptoms upon allergen exposure.

Acknowledgments

We thank Annemarie van Schadewijk, Padmini Khedoe, Anne van der Does, Simone Hermans, and Ruben van den Oever for technical assistance. Mouse FcγRII/III-binding inhibitor (2.4G2) was a kind gift of Louis Boon (Bioceros).

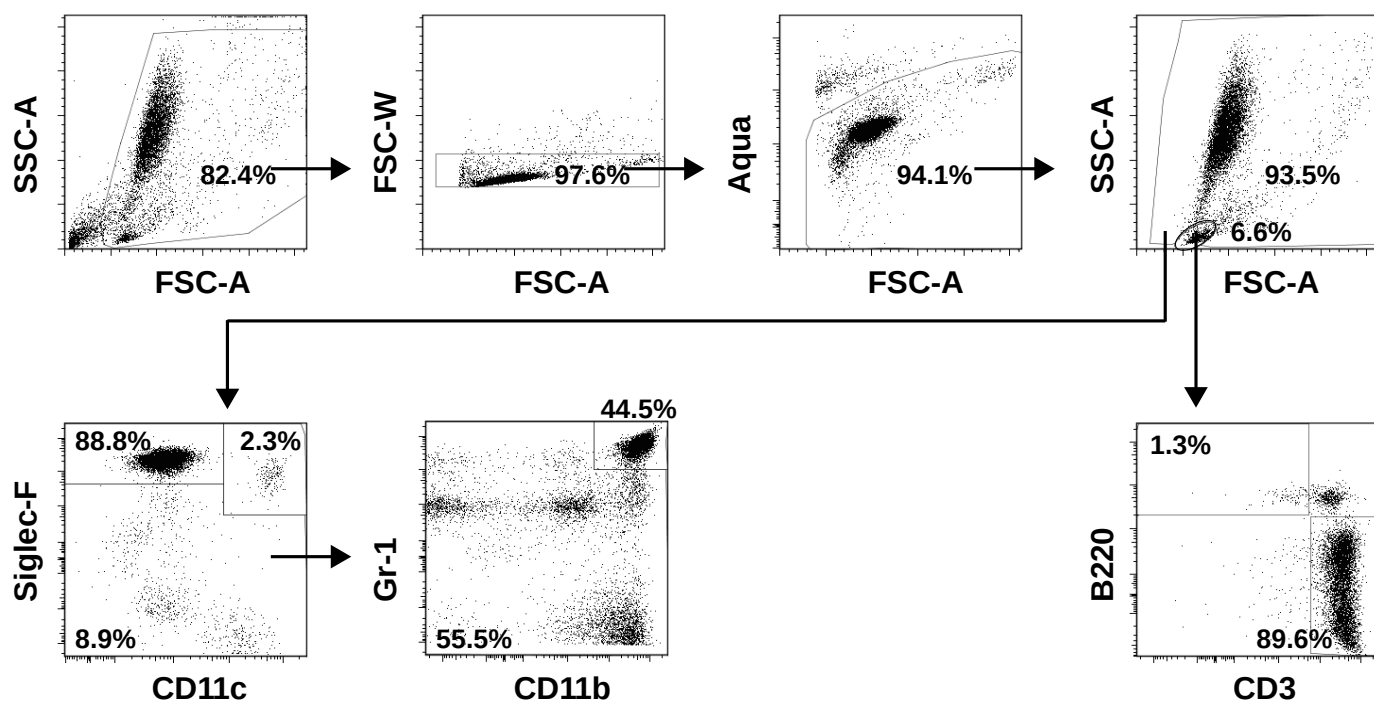
Disclosures

The authors have no financial conflicts of interest.

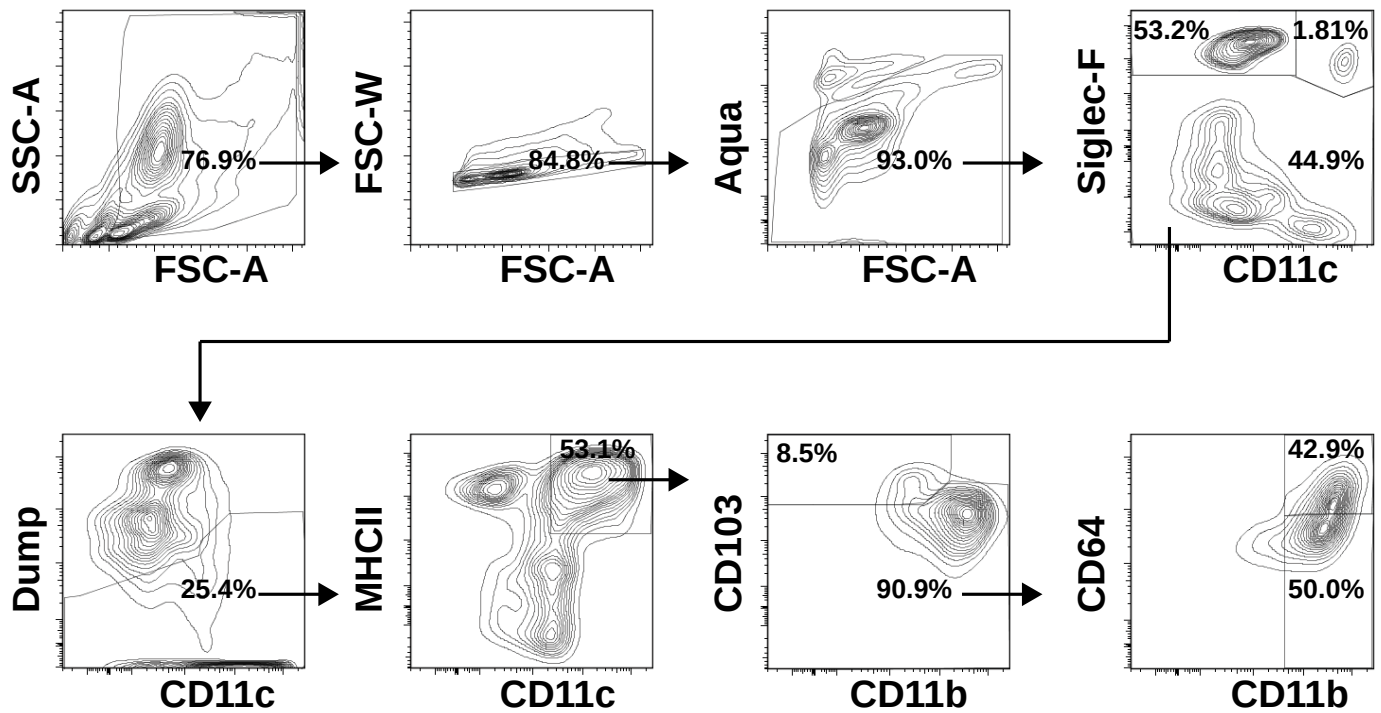
References

- Global Initiative for Asthma. 2017. Global Strategy for Asthma Management and Prevention. Available at: <http://ginasthma.org/2017-gina-report-global-strategy-for-asthma-management-and-prevention>. Accessed: July 3, 2017.
- Lundbäck, B., H. Backman, J. Lötvall, and E. Rönmark. 2016. Is asthma prevalence still increasing? *Expert Rev. Respir. Med.* 10: 39–51.
- Holgate, S. T. 2012. Innate and adaptive immune responses in asthma. *Nat. Med.* 18: 673–683.
- Wenzel, S. E. 2012. Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat. Med.* 18: 716–725.
- Barnes, P. J. 2008. Immunology of asthma and chronic obstructive pulmonary disease. *Nat. Rev. Immunol.* 8: 183–192.
- Lambrecht, B. N., and H. Hammad. 2015. The immunology of asthma. *Nat. Immunol.* 16: 45–56.
- Baldacci, S., S. Maio, S. Cerrai, G. Sarno, N. Baiz, M. Simoni, I. Annesi-Maesano, and G. Viegi, HEALS Study. 2015. Allergy and asthma: effects of the exposure to particulate matter and biological allergens. *Respir. Med.* 109: 1089–1104.
- Milligan, K. L., E. Matsui, and H. Sharma. 2016. Asthma in urban children: epidemiology, environmental risk factors, and the public health domain. *Curr. Allergy Asthma Rep.* 16: 33.
- Strachan, D. P. 1989. Hay fever, hygiene, and household size. *BMJ* 299: 1259–1260.
- Bloomfield, S. F., G. A. Rook, E. A. Scott, F. Shanahan, R. Stanwell-Smith, and P. Turner. 2016. Time to abandon the hygiene hypothesis: new perspectives on allergic disease, the human microbiome, infectious disease prevention and the role of targeted hygiene. *Perspect. Public Health* 136: 213–224.
- Marsland, B. J., A. Trompette, and E. S. Gollwitzer. 2015. The gut-lung axis in respiratory disease. *Ann. Am. Thorac. Soc.* 12(Suppl. 2): S150–S156.
- Roberts, S. E., S. Morrison-Rees, D. G. Samuel, K. Thorne, A. Akbari, and J. G. Williams. 2016. Review article: the prevalence of *Helicobacter pylori* and the incidence of gastric cancer across Europe. *Aliment. Pharmacol. Ther.* 43: 334–345.
- Varbanova, M., C. Schulz, and P. Malfertheiner. 2011. *Helicobacter pylori* and other gastric bacteria. *Dig. Dis.* 29: 562–569.
- Graham, D. Y. 2015. *Helicobacter pylori* update: gastric cancer, reliable therapy, and possible benefits. *Gastroenterology* 148: 719–731.e3.
- Taube, C., and A. Müller. 2012. The role of *Helicobacter pylori* infection in the development of allergic asthma. *Expert Rev. Respir. Med.* 6: 441–449.
- Zhou, X., J. Wu, and G. Zhang. 2013. Association between *Helicobacter pylori* and asthma: a meta-analysis. *Eur. J. Gastroenterol. Hepatol.* 25: 460–468.
- Arnold, I. C., N. Dehzad, S. Reuter, H. Martin, B. Becher, C. Taube, and A. Müller. 2011. *Helicobacter pylori* infection prevents allergic asthma in mouse models through the induction of regulatory T cells. *J. Clin. Invest.* 121: 3088–3093.
- Oertli, M., M. Sundquist, I. Hitzler, D. B. Engler, I. C. Arnold, S. Reuter, J. Maxeiner, M. Hansson, C. Taube, M. Quiding-Järbrink, and A. Müller. 2012. DC-derived IL-18 drives Treg differentiation, murine *Helicobacter pylori*-specific immune tolerance, and asthma protection. *J. Clin. Invest.* 122: 1082–1096.
- Engler, D. B., S. Reuter, Y. van Wijck, S. Urban, A. Kyburz, J. Maxeiner, H. Martin, N. Yoge, A. Waisman, M. Gerhard, et al. 2014. Effective treatment of allergic airway inflammation with *Helicobacter pylori* immunomodulators requires BATF3-dependent dendritic cells and IL-10. *Proc. Natl. Acad. Sci. USA* 111: 11810–11815.
- Plantinga, M., M. Guillems, M. Vanheerswyngheles, K. Deswarte, F. Branco-Madeira, W. Toussaint, L. Vanhoutte, K. Neyt, N. Killeen, B. Malissen, et al. 2013. Conventional and monocyte-derived CD11b(+) dendritic cells initiate and maintain T helper 2 cell-mediated immunity to house dust mite allergen. *Immunity* 38: 322–335.
- von Garnier, C., M. E. Wikstrom, G. Zosky, D. J. Turner, P. D. Sly, M. Smith, J. A. Thomas, S. R. Judd, D. H. Strickland, P. G. Holt, and P. A. Stumbles. 2007. Allergic airways disease develops after an increase in allergen capture and processing in the airway mucosa. *J. Immunol.* 179: 5748–5759.
- Veres, T. Z., S. Voedisch, E. Spies, J. Valtonen, F. Prenzler, and A. Braun. 2013. Aeroallergen challenge promotes dendritic cell proliferation in the airways. *J. Immunol.* 190: 897–903.
- Taube, C., A. Dakhama, and E. W. Gelfand. 2004. Insights into the pathogenesis of asthma utilizing murine models. *Int. Arch. Allergy Immunol.* 135: 173–186.
- Debeuf, N., E. Haspegh, M. van Helden, H. Hammad, and B. N. Lambrecht. 2016. Mouse models of asthma. *Curr. Protoc. Mouse Biol.* 6: 169–184.
- Nials, A. T., and S. Uddin. 2008. Mouse models of allergic asthma: acute and chronic allergen challenge. *Dis. Model. Mech.* 1: 213–220.
- Smits, H. H., P. S. Hiemstra, C. Prazeres da Costa, M. Ege, M. Edwards, H. Garn, P. H. Howarth, T. Jartti, E. C. de Jong, R. M. Maizels, et al. 2016. Microbes and asthma: opportunities for intervention. *J. Allergy Clin. Immunol.* 137: 690–697.
- Obieglo, K., Y. van Wijck, S. de Kleijn, H. H. Smits, and C. Taube. 2014. Microorganism-induced suppression of allergic airway disease: novel therapies on the horizon? *Expert Rev. Respir. Med.* 8: 717–730.
- Li, R., C. Cheng, S. Z. Chong, A. R. Lim, Y. F. Goh, C. Loch, D. M. Kemeny, V. Angeli, W. S. Wong, and S. Alonso. 2012. Attenuated *Bordetella pertussis* BPZE1 protects against allergic airway inflammation and contact dermatitis in mouse models. *Allergy* 67: 1250–1258.
- Nembrini, C., A. Sichelstiel, J. Kisielow, M. Kurrer, M. Kopf, and B. J. Marsland. 2011. Bacterial-induced protection against allergic inflammation through a multicomponent immunoregulatory mechanism. *Thorax* 66: 755–763.
- Stiehm, M., K. Peters, K. H. Wiesmuller, A. Bufe, and M. Peters. 2013. A novel synthetic lipopeptide is allergy-protective by the induction of LPS-tolerance. *Clin. Exp. Allergy* 43: 785–797.
- Blaser, M. J., and S. Falkow. 2009. What are the consequences of the disappearing human microbiota? *Nat. Rev. Microbiol.* 7: 887–894.
- McLoughlin, R. M., and K. H. Mills. 2011. Influence of gastrointestinal commensal bacteria on the immune responses that mediate allergy and asthma. *J. Allergy Clin. Immunol.* 127: 1097–1107; quiz 1108–1109.
- Trompette, A., E. S. Gollwitzer, K. Yadava, A. K. Sichelstiel, N. Sprenger, C. Ngom-Bru, C. Blanchard, T. Junt, L. P. Nicod, N. L. Harris, and B. J. Marsland. 2014. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat. Med.* 20: 159–166.
- Gollwitzer, E. S., S. Saglani, A. Trompette, K. Yadava, R. Sherburn, K. D. McCoy, L. P. Nicod, C. M. Lloyd, and B. J. Marsland. 2014. Lung microbiota promotes tolerance to allergens in neonates via PD-L1. *Nat. Med.* 20: 642–647.
- Bik, E. M., P. B. Eckburg, S. R. Gill, K. E. Nelson, E. A. Purdom, F. Francois, G. Perez-Perez, M. J. Blaser, and D. A. Relman. 2006. Molecular analysis of the bacterial microbiota in the human stomach. *Proc. Natl. Acad. Sci. USA* 103: 732–737.
- Brawner, K. M., R. Kumar, C. A. Serrano, T. Ptacek, E. Lefkowitz, C. D. Morrow, D. Zhi, K. R. Kyanam-Kabir-Baig, L. E. Smythies, P. R. Harris, and P. D. Smith. 2017. *Helicobacter pylori* infection is associated with an altered gastric microbiota in children. *Mucosal Immunol.* 10: 1169–1177.
- Hoffmann, F., F. Ender, I. Schmutte, I. P. Lewkowicz, J. Köhl, P. König, and Y. Laumonnier. 2016. Origin, localization, and immunoregulatory properties of pulmonary phagocytes in allergic asthma. *Front. Immunol.* 7: 107.
- Koch, K. N., M. L. Hartung, S. Urban, A. Kyburz, A. S. Bahlmann, J. Lind, S. Backert, C. Taube, and A. Müller. 2015. *Helicobacter urease*-induced activation of the TLR2/NLRP3/IL-18 axis protects against asthma. *J. Clin. Invest.* 125: 3297–3302.
- Oertli, M., M. Noben, D. B. Engler, R. P. Semper, S. Reuter, J. Maxeiner, M. Gerhard, C. Taube, and A. Müller. 2013. *Helicobacter pylori* γ-glutamyl transpeptidase and vacuolating cytotoxin promote gastric persistence and immune tolerance. *Proc. Natl. Acad. Sci. USA* 110: 3047–3052.

40. Robinson, M. W., R. Alvarado, J. To, A. T. Hutchinson, S. N. Dowdell, M. Lund, L. Turnbull, C. B. Whitchurch, B. A. O'Brien, J. P. Dalton, and S. Donnelly. 2012. A helminth cathelicidin-like protein suppresses antigen processing and presentation in macrophages via inhibition of lysosomal vATPase. *FASEB J.* 26: 4614–4627.
41. Fehlings, M., L. Drobbe, V. Moos, P. Renner Viveros, J. Hagen, M. Beigier-Bompadre, E. Pang, E. Belogolova, Y. Churin, T. Schneider, et al. 2012. Comparative analysis of the interaction of *Helicobacter pylori* with human dendritic cells, macrophages, and monocytes. *Infect. Immun.* 80: 2724–2734.
42. Fehlings, M., L. Drobbe, M. Beigier-Bompadre, P. R. Viveros, V. Moos, T. Schneider, T. F. Meyer, T. Aebischer, and R. Ignatius. 2016. Usage of murine T-cell hybridoma cells as responder cells reveals interference of *Helicobacter pylori* with human dendritic cell-mediated antigen presentation. *Eur. J. Microbiol. Immunol. (Bp.)* 6: 306–311.
43. Kool, M., T. Soullié, M. van Nimwegen, M. A. Willart, F. Muskens, S. Jung, H. C. Hoogsteden, H. Hammad, and B. N. Lambrecht. 2008. Alum adjuvant boosts adaptive immunity by inducing uric acid and activating inflammatory dendritic cells. *J. Exp. Med.* 205: 869–882.



Supplemental figure 1. Gating strategy for flow cytometry analysis of cells in the BAL fluid. Cells were Aqua stained and formaldehyde fixed. Single, alive (Aqua⁺) cells were separated into lymphocytes and non-lymphocytes based on the FSC/SSC of the different populations. In the non-lymphocyte gate, eosinophilic granulocytes (CD11c⁺Siglec-F⁺), alveolar macrophages (CD11c⁺Siglec-F⁺), and neutrophilic granulocytes (CD11b⁺Gr-1⁺) were distinguished. In the lymphocyte gate, T cells (CD3⁺) and B cells (B220⁺) were analysed.



Supplemental figure 2. Gating strategy for flow cytometry analysis of DC subtypes in lung tissue. Cells were Aqua stained and formaldehyde fixed. From single, alive (Aqua⁻) cells the eosinophils (CD11c⁻Siglec-F⁺) and alveolar macrophages (CD11c⁺Siglec-F⁺) were distinguished. A dump channel made it possible to delete T cells (CD3⁺), B cells (B220⁺), neutrophils (Gr-1⁺) and NK cells (NK1.1⁺) from the analysis. Dendritic cells (CD11c⁺MHCII⁺) were separated in CD103⁺ and CD103⁻ cells. From the CD103⁻ DC a distinction was made in CD64⁺ (monocyte derived, pro-inflammatory DCs) and CD64⁻ DCs.