Compartmentalized gut lymph node drainage dictates adaptive immune responses

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The intestinal immune system has the challenging task of tolerating foreign nutrients and the commensal microbiome, while excluding or eliminating ingested pathogens. Failure of this balance leads to conditions such as inflammatory bowel diseases, food allergies and invasive gastrointestinal infections1. Multiple immune mechanisms are therefore in place to maintain tissue integrity, including balanced generation of effector T (Teff) cells and FOXP3+ regulatory T (pTreg) cells, which mediate resistance to pathogens and regulate excessive immune activation, respectively2–4. The gut-draining lymph nodes (gLNs) are key sites for orchestrating adaptive immunity to luminal perturbations2–7. However, it is unclear how they simultaneously support tolerogenic and inflammatory reactions. Here we show that gLNs are immunologically specific to the functional gut segment that they drain. Stromal and dendritic cell gene signatures and polarization of T cells against the same luminal antigen differ between gLNs, with the proximal small intestine-draining gLNs preferentially giving rise to tolerogenic responses and the distal gLNs to pro-inflammatory T cell responses. This segregation permitted the targeting of distal gLNs for vaccination and the maintenance of duodenal pTreg cell induction during colonic infection. Conversely, the compartmentalized dichotomy was perturbed by surgical removal of select distal gLNs and duodenal infection, with effects on both lymphoid organ and tissue immune responses. Our findings reveal that the conflict between tolerogenic and inflammatory intestinal responses is in part resolved by discrete gLN drainage, and encourage antigen targeting to specific gut segments for therapeutic immune modulation.

Appropriate lymphatic trafficking of immune cells to gLNs is essential for intestinal adaptive immunity4,8 (Fig. 1a). Previous studies have mapped drainage to various gLNs along the mouse gut9–12, and described immunological differences between gLNs9,13, but, to our knowledge, the underlying cellular components and functional consequences of gut segment-specific drainage have not been systematically investigated. We sought to understand how compartmentalized lymphatic drainage of the intestinal milieu contributes to distinct immune responses towards luminal antigens. We first imaged the gut lymphatic system using 3D imaging of solvent-cleared tissue stained with an antibody against the lymphatic endothelial cell (LEC) surface marker LYVE-1. En bloc imaging exposed the lymphatic route of gLN lymphatic drainage, and encouraged antigen targeting to specific gut segments for therapeutic immune modulation.

Principal component analysis (PCA) indicated that LECs and FRCs showed differential gene expression according to the gut segment the gLNs drained (Fig. 1f). LECs from D-gLNs showed a distinct metabolic signature indicative of elevated cholesterol and lipoprotein handling as well as fatty acid utilization, probably driven by the lipid-rich lymph of the proximal small intestine (Fig. 1g). FRC gene expression profiles were affected in a more pronounced manner by location, most evidently in immune cell migration and activation pathways, which were upregulated in the C-gLN (Fig. 1h, Extended Data Fig. 3e, Supplementary Tables 5–8). Differences within the small intestine were less extreme, although FRCs from I-gLNs also showed a gene signature associated with leucocyte stimulation (Extended Data Fig. 3f, g).

Because stromal cells may influence the function of antigen-presenting cells (APCs) in gLNs14, we tested whether gut-segment-specific lymphatic drainage influenced the gene expression profiles of migratory dendritic cells. The two populations of MHCIIB+ migratory dendritic cells (CD103+ CD11b+ and CD103+ CD11b+ dendritic cells; Extended Data Fig. 3h) are highly represented in gLNs and have been implicated in tolerogenic and pro-inflammatory responses, respectively16,17. PCA revealed that dendritic cells segregated between the small and large intestine, and less so between D-gLNs and I-gLNs (Fig. 2a). Both subsets of dendritic cells showed immunological and metabolic differences according to gLN location (Fig. 2b, c, Extended Data Fig. 3i–n, Supplementary Tables 9–16). Duodenal-gLN dendritic cells showed a less pro-inflammatory signature than their colonic counterparts: D-gLN CD103+ CD11b+ dendritic cells expressed lower levels of inflammatory cytokine receptors and pathways such as interferon or interleukin-1beta (IL-1β) (Fig. 2b, Extended Data Fig. 3m); and D-gLN CD103+ CD11b+ dendritic cells were specifically enriched for Ccl22, which encodes a chemokine associated with migration of pTreg cells,
and the T<sub>reg</sub>-promoting factor Aldh1a2, which encodes a rate-limiting enzyme for retinoic acid<sup>11</sup> production from retinol (Fig. 2c, Extended Data Fig. 3), o–q). Differences in retinoic acid production capacity between small- and large-intestine gLNs were preserved in all gLNs analysed when compared to SPF mice (Extended Data Fig. 3r). These data suggest that D-gLNs are more tolerogenic environments than I- and C-gLNs, at least in part owing to favourable dendritic cell and stromal cell signatures, and that this tolerogenicity is boosted by the microbiota. Overall, each gLN appears to be poised to mount an appropriate immune response corresponding to the environment of the intestinal region that it drains.

We next investigated whether CD4<sup>+</sup> T cell fates correlated with migratory dendritic cell profiles or the presence of microbiota. Although germ-free mice harbour fewer T<sub>reg</sub> (FOXP3<sup>+</sup>) cells than control mice in the small and large intestines<sup>8</sup>, we observed no difference between germ-free and specific pathogen-free (SPF) mice in the numbers of T<sub>reg</sub> cells in gLNs (Extended Data Fig. 4a–c). T<sub>reg</sub>17 (ROR<sup>+</sup>; T<sup>+</sup>) effector cells and their corresponding suppressive ROR<sup>−</sup>T<sup>+</sup> pT<sub>reg</sub> cells can be induced by the microbiota<sup>12</sup> and germ-free mice displayed a severe reduction in ROR<sup>−</sup>T<sup>+</sup> pT<sub>reg</sub> cells in all tested gLNs, although there was no significant decrease in T<sub>reg</sub>17 cells (Fig. 2d, Extended Data Fig. 4d, e). We then tracked T cell responses to segmented filamentous bacteria (SFB)—pathobionts that elicit a strong T<sub>reg</sub>17 response and preferentially colonize the ileum and colon<sup>19,20</sup>—and found that monocolonization of germ-free mice with SFB led to enrichment of ROR<sup>−</sup>T<sup>+</sup> CD4<sup>+</sup> T cells only in the I- and C-gLNs (Fig. 2e). To test whether initial CD4<sup>+</sup> T cell polarization occurs in a compartmentalized manner, we adoptively transferred naive ovalbumin (OVA)-specific CD4<sup>+</sup> cells (CD45.1 OT-II cells) into SPF mice and analysed the activation of the transferred cells upon OVA gavage<sup>17</sup>. OT-II cells proliferated and were activated in all gLNs, with the exception of the iliac and caudal gLNs (Extended Data Fig. 4f–i).

We then assessed CD4<sup>+</sup> T cell fate and its dependence on the microbiota. Amongst the retained OT-II cells (Extended Data Fig. 5a), we observed a gradient of pT<sub>reg</sub> cell induction that declined in a proximal-to-distal manner (Fig. 2f). In germ-free mice, pT<sub>reg</sub> cell induction was decreased in all gLNs when compared to SPF mice (Fig. 2f, Extended Data Fig. 5b), despite having similar antigen access, induced proliferation and increased CD25 expression by transferred OT-II cells in upper small intestine gLNs (Extended Data Fig. 5c–g), suggesting that the less favourable dendritic cell composition in germ-free gLNs contributed to this effect. Limited antigen availability was also unlikely to explain the gradient of OT-II pT<sub>reg</sub> cells in the small intestine and C-gLNs of SPF mice, as proliferation and CD25<sup>+</sup> cell frequencies did not differ between these gLNs (Extended Data Fig. 5f, g). Furthermore, intravenous administration of OVA also resulted in a decreasing proximal-to-distal gradient of FOXP3 induction, but similar OT-II cell recovery and proliferation among gLNs (Fig. 2g, Extended Data Fig. 5h–m). By contrast, OT-II ROR<sup>−</sup>T<sup>+</sup> T<sub>reg</sub>17 and OT-II ROR<sup>−</sup>T<sup>+</sup> pT<sub>reg</sub> cells showed an ascending proximal-to-distal gradient that was independent of the microbiota (Fig. 2h, i) and correlated with an increase in IL12/23,p40<sup>+</sup> frequency among CD103<sup>+</sup>CD11b<sup>+</sup> dendritic cells (Extended Data Fig. 5n). These data indicate that under homeostatic conditions, the D-gLNs are the primary sites for induction of pT<sub>reg</sub> cells by dietary antigens. By contrast, distal gLNs favour differentiation of T<sub>reg</sub>17 and ROR<sup>−</sup>T<sup>+</sup> pT<sub>reg</sub> cells, underscoring the immunological distinctness of the proximal and distal intestinal mucosa.

We investigated the possible consequences of anatomical segregation of T cell fates along the intestine. First, we analysed T cell responses to an antigen delivered to distinct intestinal sites by surgical injection of OVA-containing solution into the duodenum or ileum. This approach did not elicit significant induction of ROR<sup>−</sup>T<sup>+</sup> pT<sub>reg</sub> cells, possibly because of the pro-inflammatory surgical setting and lack of post-inal mechanisms. Ileal injection of OVA elicited more pronounced differentiation of ROR<sup>−</sup>T<sup>+</sup> T<sub>reg</sub>17 and ROR<sup>−</sup>T<sup>+</sup> pT<sub>reg</sub> cells than duodenal injection of OVA in the respective draining gLNs; this response pattern was boosted by co-injection of cholera toxin (CT; Fig. 3a, Extended Data Fig. 6a–h). This result confirms that I- and C-gLNs have a greater pro-inflammatory potential than D-gLNs. We next injected CT–OVA into the ileum or duodenum and compared these mice to sham-operated mice for susceptibility to OVA-expressing Salmonella enterica Typhimurium (Stm–OVA), which infects the ileum and caecum before systemic dissemination. Only mice previously injected with CT–OVA in the ileum displayed delayed weight loss compared to sham-operated...
mice (Fig. 2b, c, Extended Data Fig. 6i), which correlated with reduced *Salmonella* invasion and an increased frequency of RORγ+ T cells among CD45.1+CD4+ cells in gLN+ T cell activation in the C-gLNs (Fig. 3d, e, Extended Data Fig. 6m–p), or by infection with wild-type *Citrobacter* (Extended Data Fig. 6q–u).

The gLNs are crucial for physiological responses to microbiota, pathogen-resistance mechanisms and oral tolerance[21,22]. We investigated whether the I- and C-gLNs are required for the generation of SFB-specific T cells. After transfer of naive SFB-specific CD4+ T cells (7B8tg cells) into SPF mice colonized with SFB, we recovered substantial numbers of transferred cells only from the I- and C-gLNs, which upregulated RORγ+ T expression (Fig. 3f, g, Extended Data Fig. 7a–e). Surgical removal of I- and C-gLNs shifted the SFB-specific T cell response to the gLNs that drain gut segments with lower colonization (Fig. 3f, g, Extended Data Fig. 7a–g), and this change correlated with ectopic expansion of 7B8tg cells in the intestine, but only if some SFB were present in the lumen owing to recent colonization (Extended Data Fig. 7a–c, h–s). It is unlikely that surgery caused lymph rerouting to surrounding gLNs, because dye did not spread to adjacent lymph nodes, or expansion of SFB into surrounding gut segments, as evidenced by SFB-specific 16S qPCR (Extended Data Fig. 8a–c). Hence, these data suggest that individual gLNs also modulate T cell responses in the intestinal tissue in a region-specific manner. In the presence of pathogens or pathobionts, this regionalization may help to contain tissue damage. Together, these results illustrate the beneficial effects of discrete gLN
Duodenal infection leads to a compartmentalized immune conflict in the duodenal gLNs and to compromised oral tolerance. a, Dissected gLNs from non-infected (NI) C57BL/6 mice or mice infected with 700 S. venezuelensis (Sv) larvae 8 days before dissection. b, Total CD45+ cell counts (n = 5, representative of three independent experiments). c–f, Frequency of CD103−CD11b+ (c), CD103+CD11b+ (d) and CD103−CD11b− (e) cells among MHCII+CD11c+ cells and eosinophils among CD45+ cells (f) (n = 5, representative of three independent experiments). g, Representative flow cytometry plot of GATA3+ and FOXP3+ CD4+ T cells. h, Frequency of GATA3+ CD4+ T cells (n = 5, representative of three independent experiments). i, Frequency of total FOXP3+ cells among CD45.1+ cells in gLNs 64 h after adoptive transfer of 1 × 106 naive CD45.1+ OT-II cells into CD45.2 mice (n = 8, pool of two independent experiments) infected with S. venezuelensis larvae or not infected 8 days before analysis, and gavaged with OVA 48 h and 24 h before analysis. j, Scheme of oral tolerance experimental set-up in S. venezuelensis-infected mice. k–m, Total eosinophils in BALF (k), frequency of eosinophils among CD45+ cells in lung tissue (l) and OVA-specific IgG1 levels in serum (m) from mice infected with S. venezuelensis or not infected during antigen feeding (+OVA groups) or no feeding (−OVA groups), 21 days after first immunization with OVA–alum (j) (n = 13 for +OVA groups, n = 10 for −OVA groups, pool of two independent experiments). *P < 0.05, **P < 0.01, ***P < 0.005; NS, not significant (one-tailed t-test or ANOVA).

Drainage, which may avoid immunological conflict between responses induced at different gut sites and help to preserve these responses in the tissue.

Viral and bacterial gastrointestinal infections can perturb the generation of pTreg cells that recognize dietary antigens and subsequently impair oral tolerance.6–23 We investigated whether the proximal gLNs alone are necessary or sufficient for oral tolerance, a pTreg cell-dependent process, by surgical removal of the D-gLNs or the I- and C-gLNs followed by the induction of asthma suppression mediated by an oral antigen.17 Oral tolerance was intact following the removal of proximal or distal lymph nodes (Extended Data Fig. 8d–i), probably because pTreg cells generated in remnant gLNs are sufficient to maintain tolerance.17 To induce immunological conflict only in the proximal intestine-draining gLNs, we chose the helminth Strongyloides venezuelensis, which displays distinct duodenal tropism.24 Upon infection with S. venezuelensis, only the D-gLNs were swollen, underwent significant restructuring and displayed increased immune cell counts compared to gLNs of non-infected mice, and this was consistent with compartmentalized duodenal worm load (Fig. 4a, b, Extended Data Fig. 9a–c, Supplementary Videos 8–17). We also observed a selective influx of CD11b+ dendritic cells into D-gLNs (this has been previously linked to the induction of Th2 responses25) at the expense of the more tolerogenic CD103+CD11b+ and CD8α+ dendritic cell subsets; D-gLNs also showed a type 2 immunity signature comprising eosinophils, GATA3+ Th2 cells and GATA3+ Treg cells even after the infection was cleared (Fig. 4c–h, Extended Data Fig. 9d–l). Upon infection with S. venezuelensis, transferred OT-II cells showed a significant reduction in OVA gavage-induced upregulation of FOXP3 (P < 0.01 for D1 and D2) and CD25 (P < 0.001 (D1), P < 0.05 (D2)) specifically in the D-gLNs (P < 0.01 for D1 and D2) compared to non-infected mice, despite a similar frequency of CD45.1+ cells being recovered (Fig. 4i, Extended Data Fig. 10a–e). Finally, we investigated whether transient infection during initial antigen exposure affected tolerance to dietary antigens (Fig. 4j). Mice infected with S. venezuelensis showed partial impairment of suppression of eosinophilia and dendritic cell infiltration into bronchial-alveolar lavage fluid (BALF) and lungs, and increased OVA-specific IgG1, compared to non-infected mice (Fig. 4k–m, Extended Data Fig. 10f, g). Together, these data show that duodenal infection can perturb local responses and alter systemic tolerance to gut antigens.

Our data uncover a mechanism by which the intestinal immune system simultaneously handles regulatory versus pro-inflammatory responses; by anatomical segregation of these reactions into functionally distinct gLNs. They also show that effector (Th2) cells and corresponding regulatory mechanisms (lineage-defined Treg cells)
may co-operate at every site to dampen excessive, gut segment-specific inflammation. It remains to be determined whether the presence of specific dendritic cell subpopulations with varying tolerogenic or inflammatory roles in specific GLNs mediate distinct responses to dietary, microbiota or pathogen-derived antigens. The efficient drainage of dietary antigens into tolerance-promoting lymph nodes associated with the proximal intestine suggest that these lymph nodes help to prevent food allergies, and that duodenal infection and dysbiosis can alter the allergic outcome. However, the dietary duodenal route could be exploited therapeutically for inducing tolerance to otherwise inaccessible pro-inflammatory antigen sources, such as dysbiotic bacteria in patients with inflammatory bowel diseases, while selective targeting of distal GLNs might lead to successful intestinal vaccination.

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Mice. C57BL/6J CD45.1 (B6.SJL-Ptprca Pepcb/BoyJ) mice were from Jackson laboratories, CD45.2 (C57BL/6) mice were purchased from Jackson Laboratories or Taconic Farms, and CD45.1 OT-II TCR-transgenic mice were originally purchased from Jackson ImmunoResearch Laboratories, Inc. Aqua LIVE/DEAD Fixable Aqua (Invitrogen, L34957), anti-IgE, Invitrogen, RMGE00; and IgE isotype control, eBioscience 554118. Horseradish peroxidase-conjugated streptavidin was purchased from Jackson Immunoresearch Laboratories, Inc.

For cytokine analysis in T cells, cells were incubated for 3.5 h in RPMI medium with 10% fetal bovine serum (FBS), brefeldin A (0.5 µg ml⁻¹), Sigma B7651), ionomycin (0.5 µg ml⁻¹, Sigma 10634), and phorbol 12-myristate 13-acetate (100 ng ml⁻¹, Sigma P8139); for cytokine analysis in dendritic cells, cells were incubated for 6 h in RPMI with 10% FBS and GolgiPlug (1 to 1,000, BD Biosciences 555029). Cell populations were stained with Aqua in phosphate-buffered saline (PBS), followed by incubation with Fc block and antibodies against the indicated cell surface markers in fluorescence-activated cell sorting (FACS) buffers (PBS, 1% BSA, 10 mM EDTA, 0.02% sodium azide). The cells were analysed live, or fixed in 1% PFA/PBS. For intracellular staining, cells were first stained for surface epitopes and then fixed, permeabilized and stained according to the manufacturer's protocol (eBioscience 00-5123-43). Flow cytometry was performed on an LSRII (BD Biosciences) and analysed using FlowJo software (Tree Star). Cell division index was calculated using the FlowJo formula (http://www.flowjo.com/v765/en/proliferation.html), in which the index represents the fraction of total cell divisions over the calculated total starting cells.

For tissue clearing and staining, the dISCO protocol was followed as detailed on the continuously updated website http://disco.info, with the following specifics: tissues were stained in primary and secondary antibodies for 4 days each, and were embedded in 1% agarose-TAE before final dehydration. Blocks were imaged using a LaVision UltraMicroscope II and software. Images were reconstructed using Imaris software.

For tissue isolation, cells were washed with PBS and stained with 1% paraformaldehyde, 0.1% sodium azide in PBS for 15 min. Cells were then incubated with permeabilization buffer (0.1% saponin) for 30 min at 4°C. After washing, cells were stained with appropriate antibodies and analyzed by flow cytometry.
were disrupted using 25 G needles, and Liberase TL (Roche) was added to a final concentration of 0.25 mg/ml with collagenase D (Roche) to a final concentration of 400 U/ml. Tissues were incubated at 37 °C on an orbital shaker at 80 r.p.m. for 10 min, and disrupted at room temperature by gently pipetting the digest up and down 50 times, followed by an additional 20 times. The reaction was stopped by placing the digest on ice and adding 14 ml of ice-cold digestion medium. Cells were spun down at 700g for 5 min at 4 °C, resuspended in erythrocyte lysis buffer (Sigma), 100 µl up with sodium again, and spun twice by centrifugation. Isolation of lymphocytes and APCs from small and large intestines. Intestines were separated from mesentercy, and Peyer’s patches (small intestine) and faeces were removed. For segmentation of the small intestine, the upper 25% of the small intestine was taken as duodenum, the next 50% as jejunum and the last 25% as ileum. This division allowed quick processing of multiple specimens, though it can lead to some contamination of the ileum and duodenum with jejunum. The caecum was included in the preparation of the large intestine. Intestines were cut longitudinally and washed twice in PBS. Tissue was cut into 1-cm pieces, mucus was removed by incubating the tissue again for 10 min in PBS and 1 µl DTT, and the epithelium was removed by two incubations in 25 ml of HBSS, 2% FCS and 30 mM EDTA for 10 min at 37 °C at 230 r.p.m. with vigorous shaking after each incubation. Tissues were washed in PBS over a sieve, then finely chopped and digested in 6 ml of RPMI per gut segment (Gibco), 2% FCS, 200 µg/ml DNaseI (Roche) and 2 mg/ml collagenase 8 (Gibco) for 45 min at 37 °C, 5% CO2. Digests were taken up and resuspended 10 times and passed through a sieve, and the collagenase was quantified by adding of 15 ml cold RPMI, 2% FCS. Pellets were resuspended in 40% Percoll (BD Pharmingen) complemented with RPMI, 2% FCS, passed through the same steps and separated by centrifugation. Concentration was determined using a High Sensitivity DNA Assay (Bioanalyzer). Sample quality was again verified by 1,500–2,000 bp long by a High Sensitivity DNA Assay (Bioanalyzer). Concentration and sample concentrations were measured on the Qubit fluorometer with ddH2O, and 2.5 µl of the indicated volumes. Sample quality was again verified by Bioanalyzer, and sample concentrations were measured on the Qubit fluorometer and adjusted to a concentration of 4.54 ng/µl. All samples were pooled at equal contributions and run in multiple lanes. Sequencing was performed using 76-base single-end reading on a NextSeq instrument (Illunina).

RNA-seq data analysis. Gene expression was quantified using the raw fastq files and the mouse reference transcriptome M18 (Genecode, GRCm38.p6) as input for Kalisto (v0.43.0). The resultant normalized transcript frequencies were provided to the R package Sleuth for differential analysis (v0.30.0). Analyses were conducted at the gene level, and the likelihood ratio test was applied to capture gene expression differences among all groups. When testing between two groups, the Wald test was used to determine differentially expressed genes. Genes with log-fold changes greater than 1 or less than –1 and a false discovery rate (FDR) of 0.05 were considered significant for downstream studies. Gene set enrichment analysis (GSEA) was performed by ranking genes based on log, fold changes of paired comparisons and subsequently run as a GSEA pre-ranked analysis (GSEA v3.0) with the c5.all. v6.1. symbols.gmt (Gene Ontology) gene set database. All pathways with an FDR of 0.25% or less were considered significantly different.

RALDH activity assay. RALDH activity was determined using the Aldefluor kit (STEMCELL Technologies) according to the manufacturer’s protocol. Cells were analysed by flow cytometry 30 min after the addition of substrate.

125-I-OVA labelling and biodistribution. Ligation of OVA was performed as described previously18. Mice were fasted for 3 h before gavage with 4 × 107 CPM 125-I-OVA and 50 mg cold OVA (grade III) in 200 µl PBS, and samples were taken at 1 h and 5 h post gavage. The wet weight of tissues was measured before measuring radioactivity on a gamma counter (Packard Cobra). Input radioactivity was estimated by counting 10% of the gavaged material.

Adoptive T cell transfer. Naïve CD4 T cells from spleen and lymph nodes were isolated by positive selection using biotinylated antibodies against CD8α, CD25, CD11c, CD11b, TER-119, NK1.1, and B220 and anti-biotin MACS beads (Milteny Biotec). The purity of transgenic CD4+ T cells was verified by flow cytometry (CD45.1+ V20+ V15+ CD25+) for OT-II cells, CD45.1+ V14+CD25+ for 7B2tg cells, typically >90%). T cells were labelled using the Cell Trace Violet or CFSE Cell Proliferation Kit (Life Technologies). For OT-II cells, 1 × 106 cells were transferred by retro-orbital injection under isoflurane gas anesthesia. For 7B2tg cells, 4 × 105 cells were transferred for analysis of gLN 60 h post-transfer and 5,000 cells for analysis 7 days or more after transfer in the gut and gLN.

Oral antigen administration. OVA (grade III, Sigma, A5378) was administered at 50 mg in 200 µl PBS by gavage using metal gavage needles. Two doses were given with a 24-h interval, the first dose given 16–24 h after adoptive OT-II cell transfer.

Intravenous antigen administration. Endotoxin-low OVA (Worthington) was administered by retro-orbital injection at either 1 mg or 0.1 mg in 100 µl PBS 16 h after adoptive OT-II cell transfer. The high dose corresponds to the maximum of 125-I-OVA recovered in blood upon OVA gavage (4% of 50 mg gavage per ml plasma, see Extended Data Fig. 5e, equivalent to 2% in blood, estimating the blood volume of a 20 g mouse is around 1 ml equating to 1 mg); the ten times lower dose is equivalent to dosages used in previous studies.

Intestinal CT–OV A injection. Naïve OT-II cells were adoptively transferred into 7-week-old C57Bl/6 mice 16–24 h before surgery (1 × 106 cells for T cell fate analysis in gLN, 1 × 105 for subsequent Stm-OVA infection). Mice were anesthetised subcutaneously with 100 mg/kg ketamine (controlled substance provided by the Rockefeller University animal facility), 10 mg/kg xylazine (Akorn), and in the presence of 5 mg/kg analgesic meloxicam (Putney) in 0.5 ml saline. The abdominal area was then shaved, and sterilized by three cycles of wiping with iodine solution and 70% ethanol after 20 µl of 0.25% bupivacaine (Hispura, Inc.) was injected intradermally at the prospective site of incision. The mouse was placed on a heat mat and covered by sterile surgical plastic with an opening above the abdomen. All work from here was performed aseptically. Skin was incised in the middle of the abdomen and the peritoneum was cut open. Filter-sterilized PBS solution (100 µl) containing 50 mg OVA and 5 µg cholaeris toxin or not was injected into the duodenum (5 mm after the pylorus) or the ileum (5 mm before the most distal Peyer’s patch) using a 28.5 G needle pointing towards the distal intestine. The peritoneal muscles were aligned and sutured using absorbable suture (PDS®II, Ethicon), the skin closed with autoclips (Ken Scientific Corp.), and the wound covered with Triple Antibiotic Ointment (Honeywell Safety Products, USA). Mice were allowed to fully awaken in a cage placed on a heat mat. For sham surgery, mice were opened and sutured again. Mice that had undergone different surgeries were pooled in cages. On the day after surgery, mice were monitored for agility and passing stool. Mice were treated with 0.3 mg/kg of buprinex (controlled substance) and 5 mg/kg meloxicam 24 h after the first meloxicam treatment and every 24 h for another 3 days.

Stm-OVA infection. Seven days after CT–OV A injection, mice were gavaged with 20 mg streptomycin in 200 µl PBS. On the same day, Stm-OVA was grown overnight from an overnight culture of the strain grown at 30 °C, at pH 7. On the next day, a 100 µl aliquot of Stm-OVA was sub-cultured in 1 ml LB broth. After 3.5 h, 50 µl subculture was diluted in 50 ml PBS, giving rise to a density of 107 CFU per 100 µl, and mice were immediately gavaged with 100 µl of this solution. The infection was
timed such that 24 h had elapsed since streptomycin administration and the mice had been fasted for 4 h before infection. Initial body weight was taken just before gavage. Mice were weighed every 24 h after infection. In the survival study, mice were killed when their weight reached 80% or less of their starting weight. For analysis of S. venezuelensis dissemination, mice were killed 48 h after infection, and CFU in organs was determined by serial dilutions on Salmonella Shigella agar plates. C. rodentium and Citro-ova infection. Mice were infected with 1 × 10^9 CFU of C. rodentium or Citro-ova. For Citro-ova infection, mice were kept on 100 mg/l kanamycin in drinking water to ensure the OVA-expressing plasmid was not lost. Non-infected control mice were also kept on kanamycin. Infection was monitored by plating faeces onto MacConkey agar plates. Nine days after infection, 1 × 10^9 naive OT-II cells were adoptively transferred and mice subjected to OVA gavage as described above.

SFB colonization. SFB was obtained from frozen stocks of caecal contents (kept at –80°C for less than 6 months) of mice monocolonized with SFB, which were diluted in PBS (2 ml/caecum) and passed through a 70-µm mesh. Mice were colonized by two gavages of 0.4 ml of caecal content preparation, 24 h apart. Colonization was verified by real-time PCR of faecal DNA (Quick-DNA Fecal/Soil microbe mini-prep kit, Zymo research, Cat. No. D6010). PCR was performed in the presence of Power SYBR Green PCR master mix (Applied Biosystems, 4367659) on a Quant Studio 3 PCR machine (Applied Biosystems), using SFB-specific 16S primers (fwd: GACGCTGAGGCATGAGAGCAT; rev: GACGGCACGGATTGTATTTCCA). The SFB Ct value was normalized by the Ct obtained in the PCR using universal bacterial 16S primers (fwd: ACTCCTACGGGAGGCAGCAGT; rev: ATTACCAGCGGCTGCTGGC).

Lymph node surgery. Mice were prepared and sutured and monitored postoperatively as for CT-OVA injection. To expose the ileal and caecal lymph nodes, the caecum was gently pulled out using cotton tipped applicators soaked in saline. To expose the upper duodenal lymph nodes, the duodenum was shifted to the left just under the pylorus; the same loop was shifted to the right to expose the distal duodenal LNs. Lymph nodes were removed by holding onto the lymph node with tweezers and gently pulling it while slowly and closely cutting around the node with microsurgical scissors, such that no bleeding occurred. For sham operation, the caecum was pulled out and the lymph nodes exposed for 2 min. The caecum was then placed back into its original position and the peritoneal cavity filled with 0.5 ml pre-warmed saline. Mice were allowed to recover from surgery for twelve days before colonization with SFB (Iax mice), nineteen days before adoptive transfer of 7B8tg cells, and two weeks before OVA gavage (oral tolerance).

S. venezuelensis passage and infection. S. venezuelensis was maintained in Wistar rats by subcutaneous infection with 30,000 larvae. On day 6–8 after infection, the caecum (containing eggs) was removed and spread on Whatman paper, which was placed into a beaker with water at 28°C. The hatching larvae were collected over 4 days and the cycle re-initiated. Mice were infected subcutaneously with 700 larvae per mouse. Adult worm load was assessed in total epithelial scrapes from the gut.

Alum immunization and airway challenge. Seven days after oral administration of OVA, 4 µg of endotoxin-free OVA antigen adsorbed to 40 µl Imject Alum Adjuvant (Fisher Scientific) was injected intraperitoneally in a final volume of 400 µl made up with PBS. Immunization was repeated after 7 days. To induce airway inflammation, mice were anaesthetized and intranasally administered 10 µg of sterile OVA grade VI in 50 µl PBS (25 µl per nostril) on days 14, 17 and 21 after the first intraperitoneal immunization. Total IgE was measured to confirm previous infection with S. venezuelensis (250–350 ng/ml in plasma compared to 5–10 ng/ml in plasma of uninfected mice).Bronchialveolar lavage (BAL), lung histology and infiltrate analysis by flow cytometry. Mice were anaesthetized by intraperitoneal injection of 0.35 ml 2.5% avertin (Sigma), the trachea was cannulated and lungs were lavaged once with 0.5 ml and then with 1.0 ml PBS. Total BAL cells were counted after erythrocyte lysis and stained for FACS analysis. Lungs were perfused via the right ventricle with 10 ml saline to wash out residual blood. One lobe was digested in 400 U/ml collagenase D/HBSS and processed for FACS analysis. Eosinophils were determined as CD45+SSA-CD11c-CD11b+Ly6G+SiglecF+ and dendritic cells as CD45+MHCIICD11c+CD64+SiglecF-.

Anti-OVA IgG1 and total IgE ELISAs. Enzyme-linked immunosorbent assays (ELISAs) were performed as described previously.

Statistical analysis. No statistical methods were used to predetermine sample size. Statistical analysis was performed in GraphPad Prism 7.0 software. Error bars indicate s.e.m. Multivariate data were analysed by applying one-way ANOVA and Tukey’s multiple comparison post hoc test; comparisons between two treatment conditions were analysed using one-tailed unpaired Student’s t-test. Survival curve was analysed using Mantel–Cox, Gehan–Breslow–Wilcoxon and logrank tests. A P value of less than 0.05 was considered significant.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

Source data for all figures are provided with the paper. For RNA-seq experiments the raw and processed data generated here can be obtained at the Gene Expression Omnibus database under the accession code: GSE121811.
Extended Data Fig. 1 | Extended analysis of gLN connectivity and drainage in the peritoneal cavity. a–e, 3D reconstruction of mouse lymphatics (anti-LYVE-1) after solvent clearing (iDISCO+) and light sheet microscopy of: the central chain of gLNs connected to the duodenum via afferent vessels (a), the D-gLNs with respect to the liver and pancreas (co-stained with anti-insulin; b), the distal intestine gLNs connected to the ileum (c), and individually dissected peritoneal gLNs (d) of SPF C57BL/6 mice. e, All peritoneal gLNs from germ-free and SPF C57BL/6 mice.

f–i, Fast green spreading upon injection into gLNs draining the duodenum (f), jejunum (g), ileum (h) and caecum (i) of 10-week-old mice. Pictures taken up to 15 min after dye injection.

j–m, Fast green spreading upon injection into the lymphatics in the muscularis of the duodenum (j), jejunum (k), ileum (l) and caecum (m) of 10-week-old mice. In o the arrow denotes the direction of lymph flow from intestine to gLNs; CD11c was revealed by using ItgaxVenus mice and staining against GFP.
Extended Data Fig. 2 | Imaging of intestinal lymphatic vessels and characterization of $[^{3}H]$retinol absorption along the gut of SPF and germ-free mice. a, 3D reconstruction of mouse lymphatic vessels (anti-LYVE-1) after iDISCO+ processing of villi and submucosa along the intestines of germ-free (GF) and SPF C57BL/6 mice; lymphatics protruding into the villi (lacteals) are indicated. b, Percentage of $[^{3}H]$retinol absorption into lymph (L), portal vein serum (PV) or systemic serum (S) of mice 3 h after gavage with $1 \mu Ci$ $[^{3}H]$retinol in 100 $\mu l$ olive oil, with or without pre-treatment with 5 $\mu l$ Pluronic L-81 3 h before gavage ($n = 4$ per group). c, Percentage of $[^{3}H]$retinol absorption into the duodenum (c) or duodenal gLN (d) of C57BL/6 mice 8 h after gavage with 1 $\mu Ci$ $[^{3}H]$retinol in 100 $\mu l$ olive oil with or without pre-treatment with 5 $\mu l$ Pluronic L-81 3 h before gavage ($n = 8$ or 9 per group). Data pooled from two independent experiments with 4 or 5 animals per group each. d–g, Percentage of $[^{3}H]$retinol absorption into systemic plasma (e), indicated intestinal tissue (f) or gLNs (g) from germ-free or SPF C57BL/6 mice 1 or 5 h after gavage with 1 $\mu Ci$ $[^{3}H]$retinol in 100 $\mu l$ olive oil ($n = 3$ per group). Data representative of two independent experiments. *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$ (one-tailed $t$-test or ANOVA). g, comparison of results from germ-free mice by ANOVA; s, comparison of results from SPF mice by ANOVA; $\#$, comparison at 1 h time point; $\&$, comparison at 5 h time point.
Extended Data Fig. 3 | See next page for caption.
Extended Data Fig. 3 Extended analysis of gLN stromal and dendritic cell differences according to gut segment drained. a, Flow cytometry plot showing gating of FRCs and LECs for RNA-seq. Pre-gates indicated above. b–g, Some differentially regulated pathways among LECs sorted from duodenal versus colonic gLNs (NES, normalized enrichment scores) (b); differentially expressed genes among LECs sorted from ileal versus colonic gLNs or ileal versus duodenal gLNs (c, d); some differentially regulated pathways among FRCs sorted from duodenal versus colonic gLNs (e); differentially expressed genes among FRCs sorted from ileal versus colonic gLNs or ileal versus duodenal gLNs (f, g), all from SPF C57BL/6 mice and identified by RNA-seq. Blue, metabolism-related pathways or genes; red, immunity-related pathways or genes. h, Flow cytometry plot showing gating of CD103+CD11b+ and CD103+CD11b− dendritic cells for RNA-seq. Pre-gates indicated above. i–l, Differentially expressed genes identified by RNA seq among CD103+CD11b+ (i, k) and CD103+CD11b− (j, l) dendritic cells sorted from ileal versus colonic gLNs or ileal versus duodenal gLNs from SPF C57BL/6 mice. m, n, Some differentially regulated pathways among CD103+CD11b+ (m) and CD103+CD11b− (n) dendritic cells sorted from duodenal versus colonic gLNs. o–q, Mean fluorescence intensity of fluorescein isothiocyanate-positive boron-dipyrromethene-tagged aminoacetate (Aldefluor) in CD103+CD11b− (o), CD103+CD11b+ (p), and CD8α+ or CD11b+ (q) dendritic cells from gLNs from germ-free and SPF C57BL/6 mice (n = 3) assessed by flow cytometry 30 min after the addition of substrate. r, Ratio of CD103+CD11b+ to CD103+CD11b− dendritic cells in gLNs from SPF and germ-free mice (n = 4, representative of two independent experiments). *P < 0.05, **P < 0.01, ***P < 0.005; ns, not significant (one-tailed t-test or ANOVA).
Extended Data Fig. 4 | Extended analysis of CD4+ T cells in the different gLNs of SPF and germ-free mice and activation of OT-II CD45.1 cells in all gLNs upon OVA gavage. a–e, Frequency of TCRβ+CD4+ cells among CD45+ cells (a), FOXP3+NRP1+ (b) and FOXP3+NRP1- cells (c) among TCRβ+CD4+ cells, RORγT+ cells among FOXP3+NRP1- cells (d) and FOXP3-RORγT+ cells among TCRβ+CD4+ cells (e) in indicated gLNs from germ-free or SPF C57BL/6 mice (n = 4). Data representative of two independent experiments. f–i, Frequency of CD25+ cells among CD45.1+ cells (f) and CD45.1+ cells among TCRβ+CD4+ cells (g), total CD45.1+ cells (h), and representative CFSE (carboxyfluorescein diacetate succinimidyl ester) dilution histogram (i) in indicated gLNs 64 h after adoptive transfer of 1 × 10⁶ naive CD45.1+ OT-II cells into SPF CD45.2 C57BL/6 host mice (n = 3), after gavage of OVA 48 h and 24 h before analysis.
Extended Data Fig. 5 | Extended analysis of OT-II CD45.1 and dendritic cells upon OVA administration in different gLNs of SPF and germ-free mice. a, b, Frequency of CD45.1+ cells among TCRβ+ CD4+ cells (a) and flow cytometry plots for gLN FOXP3 and CD25 expression (b) in indicated gLNs 64 h after adoptive transfer of 1 × 10^6 naive CD45.1+ OT-II cells into germ-free and SPF CD45.2 C57BL/6 host mice (n = 4) and after gavage of OVA 48 h and 24 h before analysis. c–e, 125I recovery in indicated gLNs (c), intestinal tissue (d) and plasma (e) of germ-free and SPF C57BL/6 mice (n = 3 per time point and group), 1 h or 5 h after gavage with 4 × 10^6 CPM 125I-OVA in 50 mg cold OVA. f, g, Division index of CD45.1+ cells (f) and frequency of CD25+ cells among CD45.1+ cells (g) in indicated gLNs as in a, b, h–m, Frequency of CD45.1+ cells among TCRβ+ CD4+ cells (h), division index of CD45.1+ cells (i), total CD45.1+ cell count (j), frequency of FOXP3hi cells among CD45.1+ cells (k), representative flow cytometry plot for FOXP3hi and total FOXP3 gating amongst CD3+ CD4+ cells in D-gLN (l) and frequency of FOXP3hi cells among CD45.2+ TCRβ+ CD4+ cells (m) in indicated gLNs or spleen, 64 h after adoptive transfer of 1 × 10^6 naive CD45.1+ OT-II cells into SPF CD45.2 C57BL/6 host mice (n = 3) and after intravenous injection of OVA 48 h before analysis. n, Frequency of IL-12/23 p40+ cells among CD103+ CD11b+ dendritic cells in indicated gLNs of germ-free and SPF C57BL/6 mice (n = 4 per group). #, fewer than 200 cells were recovered.

*P < 0.05, **P < 0.01, ***P < 0.005; ns, not significant (one-tailed t-test or ANOVA).
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | Extended analysis of OT-II CD45.1 cell seeding and fate in gLNs upon intestinal OVA injection or infection.

a–h, Frequency of FOXP3$^+$ ROR$\gamma$T$^+$ (a), FOXP3$^+$ ROR$\gamma$T$^-$ (b), FOXP3$^-$ ROR$\gamma$T$^+$ (c), FOXP3$^-$ CD25$^+$ (d), or FOXP3$^-$ CD25$^-$ (e) cells among CD45.1$^+$ OT-II cells, frequency of CD45.1$^+$ OT-II cells among TCR$\beta^+$ CD4$^+$ cells (f), division index (g) and total TCR$\beta^+$ CD4$^+$ cell counts (h) in indicated gLNs of mice 48 h after duodenal or ileal injection with OVA or OVA–CT, performed 16 h after adoptive transfer of $1 \times 10^6$ naive OT-II cells into SPF CD45.2 C57BL/6 host mice ($n$ = 3). i–l, Survival curve (sham $n$ = 5, duo $n$ = 6, ile $n$ = 8; i), and frequency in ileal lamina propria of FOXP3$^+$ ROR$\gamma$T$^+$ CD45.1$^+$ (j), FOXP3$^-$ ROR$\gamma$T$^+$ CD45.1$^+$ (k) and FOXP3$^-$ ROR$\gamma$T$^-$ CD45.1$^+$ (l) cells among TCR$\beta^+$ CD4$^+$ cells ($n$ = 4 per group) of mice infected with Stm-OVA 9 days after duodenal or ileal injection with OVA–CT versus sham operation, and 10 days after adoptive transfer of $1 \times 10^6$ naive OT-II cells into SPF CD45.2 C57BL/6 host mice. j–l, Intestines removed 48 h after infection. *$P < 0.05$ (Mantel–Cox and Gehan–Breslow–Wilcoxon, i), **$P < 0.005$ logrank, i), *$P < 0.05$ (ANOVA, j–l). m–p, Flow cytometry plot of CD25$^+$ and CFSE (m), frequency of CD451$^+$ OT-II cells among TCR$\beta^+$ CD4$^+$ cells (n), CD451$^+$ division index (o) and total CD451$^+$ cell counts (p) in indicated gLNs 64 h after adoptive transfer of $1 \times 10^6$ naive CD451$^+$ OT-II cells into SPF CD45.2 C57BL/6 mice infected with Citro–OVA 9 days earlier, and after gavage with OVA or PBS 48 h and 24 h before analysis ($n$ = 5). Data representative of two independent experiments. q–u, Frequency of total FOXP3$^+$ (q), FOXP3$^-$ CD25$^+$ (r), and FOXP3$^+$ ROR$\gamma$T$^+$ CD451$^+$ (s) cells among CD451$^+$ cells, frequency of CD451$^+$ cells among TCR$\beta^+$ CD4$^+$ cells (t), and frequency of FOXP3$^+$ ROR$\gamma$T$^+$ CD45.2$^+$ cells among TCR$\beta^+$ CD4$^+$ cells (u) in indicated gLNs 64 h after adoptive transfer of $1 \times 10^6$ naive CD451$^+$ OT-II cells into SPF CD45.2 C57BL/6 host mice infected or not with Citrobacter 9 days earlier, and after gavage with OVA 48 h and 24 h before analysis ($n$ = 5). ns, not significant in ANOVA or one-tailed $t$-test comparing gLNs of infected versus non-infected mice. *$P < 0.05$, **$P < 0.01$, ***$P < 0.005$ (ANOVA); $^{*}P < 0.05$ ($t$-test).
Extended Data Fig. 7 | See next page for caption.
Extended Data Fig. 7  |  Extended analysis of CD45.1+ 7B8tg cell fate in gLNs and gut upon removal of distal gLNs.  a–c, Representative flow cytometry plots of RORγT+ and CD45.1+ cell frequency among CD3+CD4+ cells in indicated gLNs of sham or ΔicLN mice as quantified in Fig. 3g (a), and frequency of Vβ14+CD45.1+ cells among CD3+CD4+ cells (b) and RORγT+ cells among Vβ14+CD45.1+ (c) cells in indicated gLNs of mice with sham operation (n = 12) or surgical removal of the I- and C1-gLNs (ΔicLN, n = 14) 64 h (day 3, Fig. 3f) after adoptive transfer of 4 × 10^5 naive SFB-specific CD45.1+ 7B8tg cells into recently SFB-colonized SPF CD45.2 C57BL/6 Jax mice. Graph represents pooled data from three independent experiments with n = 4–5 per group each.

d, e, Frequency of Vβ14+CD45.1+ cells among CD3+CD4+ cells (d) and RORγT+ cells among Vβ14+CD45.1+ cells (e) in indicated gLNs of Taconic sham or ΔicLN mice (n = 4) 64 h after adoptive transfer of 4 × 10^5 naive SFB-specific CD45.1+ cells. Note that SFB is a stable member of the microbial community in Taconic C57BL/6 mice through parental transmission.

f, g, Total numbers of CD45+ cells in C2-gLN of recently SFB-colonized SPF mice (f) or Taconic mice (g) at point of harvest.

h–n, Frequency of Vβ14+CD45.1+ IL17a+ (h), Vβ14+CD45.1+ RORγT+ (i), or Vβ14+CD45.1+ cells (j) among CD3+CD4+ cells, and of RORγT+ cells among Vβ14+CD45.1+ cells (k) and IL17a+ cells among RORγT+ Vβ14+CD45.1+ cells (l) in lamina propria of indicated gut segments; frequency of Vβ14+CD45.1+ cells among CD3+CD4+ cells (m) and RORγT+ cells among Vβ14+CD45.1+ cells (n) in gLNs of sham or ΔicLN Jax mice (n = 4) 7 days after adoptive transfer of 5,000 naive CD45.1+ 7B8tg cells into recently SFB-colonized SPF CD45.2 C57BL/6 Jax mice.

o–s, Frequency of Vβ14+CD45.1+ cells among CD3+CD4+ cells (o), RORγT+ cells among Vβ14+CD45.1+ cells (p) and IL17a+ cells among RORγT+ Vβ14+CD45.1+ cells (q) in indicated lamina propria, or of Vβ14+CD45.1+ cells among CD3+CD4+ cells (r) and RORγT+ cells among Vβ14+CD45.1+ cells (s) in gLNs of sham or ΔicLN Taconic mice 7 days after adoptive transfer of 5,000 naive CD45.1+ 7B8tg cells (n = 4). Hash, fewer than 200 cells were recovered. *P < 0.05, **P < 0.01, ***P < 0.005 (one-tailed t-test or ANOVA); ns, not significant.
Extended Data Fig. 8 | Effect of selective gLN removal on lymph flow, SFB colonization and oral tolerance. 

a, Fast Green tracing of ileal lymphatic drainage to the gLNs of SPF C57BL/6 mice, 5 min after injection of 3 µl Fast Green in sham-operated (left, biological duplicates; Extended Data Fig. 2g) or ΔicLN (middle and right, biological triplicates) mice 3 weeks after surgery. Red arrows, sites of injection. 

b–i, Relative quantification of SFB-specific 16S in luminal contents of indicated gut segments from recently SFB-colonized SPF C57BL/6 Jax mice (b, n = 13 per group from four independent experiments) or parentally colonized Taconic mice (c, n = 7 per group from four independent experiments) after sham or ΔicLN surgery. 

d–i, Total eosinophils (d) and dendritic cells (e) in BALF and frequency of eosinophils (f) and dendritic cells (g) among CD45+ cells in lungs, and total IgE (h) and anti-OVA IgG1 (i) in serum from SPF C57BL/6 mice subjected to oral tolerance as in Fig. 4k but without S. venezuelensis infection. 14 days after sham surgery (n = 7), I- and C-gLN removal (ΔicLN, n = 5) or D-gLN removal (ΔdLN, n = 6). Data are representative of two independent experiments. ns, not significant (ANOVA).
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | Extended analysis of gLN swelling and restructuring, and dendritic cell and CD4+ T cell subset frequencies, upon S. venezuelensis infection. 
a. Indicated gLN positions in non-infected (N.I.) SPF C57BL/6 mice or mice infected with 700 S. venezuelensis (S.v.) larvae 8 days earlier. 

b. 3D reconstruction of vasculature (anti-CD31) after solvent clearing (iDISCO+) and light sheet microscopy of indicated gLNs from non-infected SPF C57BL/6 mice or SPF C57BL/6 mice infected with 700 S. venezuelensis larvae 14 days earlier; scale bars, 500 µm.

c. Quantification of mature S. venezuelensis worms in gut epithelium 8 days after infection with 700 larvae. n = 8, pooled from two independent experiments.

d–g. Frequency of CD11c+ cells among CD45+ cells (d), and CD11c+ (e) and CD8α+ (f) cells among MHCIIintCD11c+ cells, or GATA3+FOXP3+ cells among TCRβ+CD4+ cells (g) in non-infected SPF C57BL/6 mice or mice infected with 700 S. venezuelensis larvae 8 days earlier; n = 5. Data are representative of three independent experiments. 

h–l. Frequency of CD103+CD11b+ (h), CD103+CD11b– (i), or CD103–CD11b+ cells (j) among MHCIIintCD11c+ dendritic cells, and of GATA3+FOXP3– (k) or GATA3+FOXP3+ (l) cells among TCRβ+CD4+ cells in denoted gLNs of SPF C57BL/6 mice infected with 700 S. venezuelensis larvae 21–50 days earlier, as indicated (n = 4 per time point) or non-infected (day 21) SPF C57BL/6 mice. *P < 0.05, **P < 0.01, ***P < 0.005; ns, not significant (one-tailed t-test or ANOVA).
Extended Data Fig. 10 | Extended analysis of OT-II CD45.1 cell seeding, activation and fate and oral tolerance upon S. venezuelensis infection.

a, Representative flow cytometry plot of FOXP3+ and CD25+ CD45.1 OT-II cells in gLNs 8 days after infection (or not) of mice with S. venezuelensis larvae. b–e, Frequency of total FOXP3–CD25+ cells among CD45.1+ cells (b) and CD45.1+ cells among TCRβ+CD4+ cells (c), total CD45.1+ cells (d) and division index (e) in indicated gLNs 64 h after adoptive transfer of 1 × 10⁶ naive CD45.1+ OT-II cells into CD45.2 SPF C57BL/6 mice (n = 4) infected (or not) with S. venezuelensis for 8 days and gavaged with OVA 48 h and 24 h before analysis. Data are representative of two independent experiments. f, g, Total dendritic cells in BALF (f) and frequency of dendritic cells among CD45+ cells in lung tissue (g) from non-infected SPF C57BL/6 mice or mice infected with S. venezuelensis during antigen feeding (+OVA groups) or no feeding (–OVA groups), 21 days after first immunization with OVA–alum (n = 13 for +OVA groups, n = 10 for –OVA groups). Data are pooled from two independent experiments. *P < 0.05, **P < 0.01, ***P < 0.005; ns, not significant (one-tailed t-test or ANOVA).
Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

| n/a | Confirmed |
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|     | - The exact sample size \( n \) for each experimental group/condition, given as a discrete number and unit of measurement |
|     | - An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
|     | - The statistical test(s) used AND whether they are one- or two-sided |
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|     | - Clearly defined error bars |
|     | - State explicitly what error bars represent (e.g. SD, SE, CI) |

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

| Data collection | N/A |
|-----------------|-----|
| Data analysis   | Flow Jo 8.7 and Flow Jo 10 (Flow Cytometry analysis); Gaph Pad Prism 7 and 8 (Graphs, statistics); R, DESeq2, GSEA v3.0 with the c5.all.v6.1.symbols.gmt (Gene Ontology) for RNA-seq analysis. |

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Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

A link to the RNAseq data will be made available prior to publication.
Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

### Sample size
A minimum of biological triplicates was used, the minimum needed to be able to perform statistics. For in vivo studies, the maximum number of animals available per treatment group were used to counteract biological variation: for all adoptive transfer experiments up to 5 animals per group, for oral tolerance experiments up to 8 animals per group, for infection and surgery experiments up to 8 per group. For surgeries (lymph node removal and intestinal antigen injection) removal surgery and OVA+ cholera toxin surgery group sizes were larger than sham (+2 mice) in the event an animal had to be euthanized during post-operative care due to the higher invasiveness of the procedure. All surviving animals were included in the study. This could lead to unequal group sizes and is indicated in the respective figure legends.

### Data exclusions
No data were excluded except if technical reason applied: for lamina propria lymphocyte populations if the preparation was more than 60% dead; no sample was recovered; for transferred cells, if fewer than 200 cells were recovered during flow cytometry (indicated throughout figure legends by #), as gates for very low frequency cells inaccurate at such low number.

### Replication
All attempts at replicating data were successful. In experiments where variation is inherent to the assay, data from independent experiments were pooled as shown in the legends (Fig. 3b, g; Fig. 4j, l-n; Extended Data Fig. 7b, c; Extended data Fig. 8b,c; Extended data Fig. 10 e, f).

### Randomization
For all studied B6 mice were purchased in batches of 10-20 mice and randomly distributed to cages. Infection/colonization studies: Cages of 5 mice were randomly infected/colonized with SFB or not. Because of the high chance of contamination it was not possible to house infected/colonized and non-infected mice in a single cage. CT-OVA injection: Mice were housed in cages of 4-5 mice and within a cage randomly subjected to sham, duodenal or ilial OVA or OVA-CT injection but ensuring mixed groups were present in each cage (2 vs 2 or 2 vs 3 or 2 vs 2 vs 1). Surgery: Mice were housed as 5 mice/cage, and each cage housed 2 vs 3 or 3 vs 2 sham operation versus lymph node removal surgery mice, to avoid any potential microbial drift or behavioral changes due to the different treatments affecting the study outcome.

### Blinding
Blinding was not possible in this study as the experimenters treating the mice were the same as those analyzing the data. The treatment groups had to be clearly identified throughout the study to prevent cross contamination (gnotobiotic/infection experiments), to be able to draw conclusions (CT-OVA injection in duodenum versus ileum) or were obvious by their anatomy (lymph nodes were removed). However, statistics were performed in an unbiased manner.

Reporting for specific materials, systems and methods

### Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | Unique biological materials |
| ☑   | Antibodies |
| ☑   | Eukaryotic cell lines |
| ☑   | Palaeontology |
| ☑   | Animals and other organisms |
| ☑   | Human research participants |

### Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | ChIP-seq |
| ☑   | Flow cytometry |
| ☑   | MRI-based neuroimaging |

Unique biological materials

Policy information about availability of materials

Obtaining unique materials

C57BL/6J CD45.1 (B6.SJL-Ptprca Pepcb/BoyJ) were from Jackson laboratories, CD45.2 (C57BL/6) mice were purchased from the Jackson Laboratories or Taconic Farms, and CD45.1 OT-II TCR-transgenic were originally purchased from Taconic Farms and maintained in our facilities. B6.Cg-Tg(Itgax-Venus)1Mnz/J mice were provided by M. Nussenzweig (The Rockefeller University). C57BL/6-Tg(Tcra,Tcrb)2Litt/J (7B8tg) mice were either purchased from Jackson Laboratories or provided by D. Littman (NYU). All mice are commercially available from the indicated suppliers.
Antibodies

**Antibodies used**

Fluorescent-dye-conjugated antibodies were purchased from BD (USA) (anti-CD45.2, 560693; anti-CD31, 557355; anti-CD103, 557495; anti-ly-6C, 560595; anti-NK1.1, 562921; anti-SiglecF, 552126; anti-RORγt, 562894; anti-IL17A, 56022 and 559502; anti-Vβ14, 553258), ebioscience (USA) (anti-CD20, 48-0452-82; anti-CD3e, 48-0031-82; anti-CD4, 83-0042-42; anti-CD8, 56-0081-82; anti-CD25, 17-0251-82; anti-CD11b, 47-0112-82; anti-CD11c, 25-0114-82 and 56-0114-82; anti-CD24, 48-0242; anti-CD45, 25-0451-82; anti-CD45.1, 25-0453-82; anti-CD69; anti-FoxP3, 12-5773-82; anti-GATA3, 12-9966-42; anti-i-A/I-E (MHCII), 46-5321-82 and 56-5321-82; anti-Ly6G, 48-5931-82; anti-Podoplanin, 25-5381-82; anti-Vaz, 48-5812-82, and Streptavidin, 46-4317-82), or Biolegend (USA) (anti-CD8a, 100744; anti-CD11b, 101236; anti-CD64, 139306; anti-TCR, 109220; anti-TER119, 116206). Additional antibodies were purchased from BioXcell and labelled in-house (anti-CD4, BE0001-1; anti-CD8a, BE0004-1; anti-CD11b, BE007; anti-CD19, BE0150; anti-TCR, BE0102). Biotinylated antibodies were purchased from BD Pharmingen (anti-B220, 553086; anti-CD8a, 553029; anti-CD11b, 553309; anti-CD11c, 553800; anti-CD25, 55370; and anti-NK1.1, 553163; anti-TER-119, 553672) or as follows: anti-IgG1, Bethyl, A90-105B; anti-IgG2c, Bethyl, A90-136B; anti-IgE, eBioscience, 13-5992-82; and anti-Neuropilin, R&D Systems, BAF566. Unconjugated antibodies used were anti-ly-6E, R and D Systems AF2125; anti-GFP (Aves Labs, GFP-1020); anti-OVA IgG1, Biolegend, 520501; anti-IgE, Invitrogen, RMGE00; IgE isotype control, eBioscience 554118. Horse-dosh peroxidase conjugated Streptavidin was purchased from Jackson Immunoresearch Laboratories, Inc. Aqua Live/Dead® Fixable Aqua Dead Cell Stain Kit, L-34965, Cell Trace CFSE and Violet Cell Proliferation kits (C34554 and C34557) were purchased from Life Technologies.

**Validation**

All antibodies are commercially available and have been validated by suppliers and previous publications.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

| Laboratory animals | C57BL/6 males, age 7-15 week were used. Age is specified in legends where necessary. |
| Wild animals | N/A |
| Field-collected samples | N/A |

Flow Cytometry

Plots

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

**Sample preparation**

Lymphocyte and APC isolation from lymph nodes. Tissues were dissected into cold HBSS, supplemented with Mg2+ and Ca2+, finely chopped and incubated in 400 U/ml Collagenase D (Roche) in HBSS for 25 min at 37°C, 5% CO2. Collagenase was quenched on ice by addition of final 10% FCS. Single cell suspensions were extracted from connective tissue by taking up and resuspending the digests five times. Erythrocytes were lysed by incubation in erythrocyte lysis buffer (Sigma) for 7 min at RT.

Stromal cell isolation from gLN. gLN were dissected into 500 µl cold RPMI, supplemented with 2% FCS and HEPES (dissection medium). Tissues were disrupted using 25 G needles, and Liberase TL (Roche) was added to a final concentration of 0.25 mg/ml, collagenase D (Roche) to a final concentration of 400 U/ml. Tissues were incubated at 37°C on an orbital shaker at 80 rpm for 10 minutes, disrupted at RT by gently pipetting digest up and down 50 times, followed by an additional 20 times. Reaction was stopped by placing digest on ice and adding 14 ml of ice cold dissection medium. Cells were spun down at 700 g for 5 minutes at 4°C, resuspended in erythrocyte lysis buffer (Sigma) topped up with medium again, spun and then subjected to staining.

Lymphocyte and APC isolation from small and large intestine. intestines were separated from mesentery, and Peyer’s Patches (small intestine) and feces were removed. For segmentation of the small intestine, the upper 25% of the small intestine were taken as duodenum, the next 50% as jejunum and the last 25% as ileum. The cecum was included in the preparation of the large intestine. Intestines were cut longitudinally and washed twice in PBS. Tissue was cut into 1 cm pieces, mucus was removed by incubating the tissue for 10 min in PBS and 1 mM DTT, and the epithelium removed by two incubations in 25 ml of HBSS + 2% FCS + 30 mM EDTA for 10 min at 37°C at 230 rpm with vigorous shaking after each incubation. Tissues were washed in PBS over a discontinuous Percoll gradient (80%/40%) at 1000 g for 25 min at room temperature (RT). APCs and lymphocytes were isolated from the interphase, washed, and stained for FACS analysis or subjected to re-stimulation.
| Instrument   | LSR II       |
|--------------|-------------|
| Software     | FlowJo 8.7  |

### Cell population abundance
For flow cytometry analysis of mLNs a minimum of 500 cells per final gate, or in the case of adoptive transfer, of adoptively transferred cells, was acquired. For lamina propria, a minimum of 200 adoptively transferred cells was acquired. Wherever this was not possible (low recovery of adoptively transferred cells) this is reported in the legend as "#, fewer than 200 cells were recovered".

### Gating strategy
All samples were pre-gated as singlet, live (Aqua negative) events. Further gates for lymphocytes are indicated in the axes or legends of each figure, but typically were events out of CD45+, CD45.1+ or TCRb+CD4+. Final gating strategy for DC subsets is in ED Fig. 3f. Stromal cells were gated as CD45-, TCRb-B220-(lineage, LIN)TER119-CD24, final gating strategy for subsets in ED Fig. 3a.

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.