Neonatally imprinted stromal cell subsets induce tolerogenic dendritic cells in mesenteric lymph nodes

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Gut-draining mesenteric lymph nodes (mLNs) are important for inducing peripheral tolerance towards food and commensal antigens by providing an optimal microenvironment for de novo generation of Foxp3+ regulatory T cells (Tregs). We previously identified microbiota-imprinted mLN stromal cells as a critical component in tolerance induction. Here we show that this imprinting process already takes place in the neonatal phase, and renders the mLN stromal cell compartment resistant to inflammatory perturbations later in life. LN transplantation and single-cell RNA-seq uncover stably imprinted expression signatures in mLN fibroblastic stromal cells. Subsetting common stromal cells across gut-draining mLNs and skin-draining LNs further refine their location-specific immunomodulatory functions, such as subset-specific expression of Aldh1a2/3. Finally, we demonstrate that mLN stromal cells shape resident dendritic cells to attain high Treg-inducing capacity in a Bmp2-dependent manner. Thus, crosstalk between mLN stromal and resident dendritic cells provides a robust regulatory mechanism for the maintenance of intestinal tolerance.
Immediately after birth, extensive colonization of body surfaces including the intestinal mucosa begins, a process that continues through childhood until a stable microbiota is established. Microbiota contribute to the hosts’ health by occupying niches for pathogenic microbes, breaking down indigestible material, synthesizing vitamins, and providing an important stimulus for the development and maturation of the mucosal and systemic immune system. Multiple immune mechanisms are in place shortly after birth to tolerate the high tide of bacterial antigens, resulting in a temporally limited reaction of the immune system towards microbiota, but at the same time increasing the susceptibility of newborn infants to infection.

Foxp3+ regulatory T cells (Tregs) play a central role in immune tolerance to self and foreign antigens. The majority of Foxp3+ Tregs are generated during thymic T cell development. However, the peripheral Treg pool is expanded for specificities towards food antigens and microbiota by peripheral conversion of conventional Foxp3+ CD4+ T cells into Foxp3+ Tregs (peripherally-induced Tregs, pTregs). The generation of pTregs most efficiently takes place within gut-draining lymph nodes (mLNs), including mesenteric LNs (mLNs). Subsequently, these pTregs migrate to and expand within the intestinal lamina propria, a process supported by microbiota and their metabolic products. This division of labor between LNs as the initiation venue of expansion, requires stably compartmentalized cellular and molecular machinery.

An increasing body of evidence suggests that the unique tolerogenic properties of mLNs are not only shaped by tissue-derived migratory CD103+ dendritic cells (DCs), but also by resident LN stromal cells (SCs), including fibroblastic stromal cells (FSCs) which dominate the T cell zone. FSCs isolated from mLNs express high levels of the retinoic acid (RA)-synthesizing enzyme retinal aldehyde dehydrogenase 2 (Aldh1a2), and thereby contribute to the tolerogenic, Treg-inducing micromilieu. Furthermore, FSCs are important for LN architecture by serving as structural scaffolds and by providing survival factors for migrating lymphocytes and DCs, thereby participating in the orchestration of appropriate cell–cell interactions required for the initiation of adaptive immune responses. This intimate interaction suggests that FSCs are critically involved in the modulation of immune responses, best evidenced by the finding that they can attenuate acute inflammatory T cell responses. However, little is known how FSC subsets functionally differ across LNs draining diverse tissues.

Reciprocal LN transplantations, where only graft-derived SCs are retained in the transplanted LN, provide valuable tools for analyzing the contribution of LN SCs to LN-specific immune responses. Using this technique we recently demonstrated that LN SCs critically contribute to the high Treg induction within mLNs and stably maintain their tolerogenic properties in a skin-draining environment. Importantly, efficient Treg induction within mLNs relies on antigen presentation by DCs, and recent studies have proposed crosstalk between LN SCs and DCs, including the responsiveness of DCs to environmental cues derived from the SC compartment. However, it is only incompletely understood which functional properties of DCs are educated by mLN SCs.

Here we report that mLN SCs are imprinted for a high Treg-inducing capacity soon after birth, and once established can stably maintain their tolerogenic potential subsequent to gastrointestinal infection and inflammation. Microbiota are critically required to imprint tolerogenic properties into mLN FSCs across different subsets, ensuring perpetuation of intestinal tolerance throughout life. Once imprinted, LN SCs can instruct LN-resident DCs (resDCs) to foster efficient Treg induction. Together, these observations reveal a mechanism whereby neonatally imprinted mLN SCs contribute to lifelong homeostatic intestinal tolerance by constantly modulating functional properties of resDCs.

**Results**

**mLN SCs show inflammation-resistant tolerogenic properties.** We previously demonstrated that mLN SCs contribute to the high Treg-inducing properties of mLNs. To collect further evidence for the stability of the tolerogenic phenotype of mLN SCs, mLNs were transplanted into the popliteal fossa of recipient mice after excision of the endogenous popliteal LN. Skin-draining LNs (pLNs) were transplanted as controls. Fifty weeks after transplantation, the Treg-inducing capacity of transplanted mLNs was analyzed by adoptive transfer of T cell receptor (TCR)-transgenic, ovalbumin (Ova)-specific naïve Foxp3+ CD4+ T cells, followed by systemic intravenous application of Ova peptide (Fig. 1a). At day 3 after antigen application, flow cytometric analysis revealed a comparable proliferation of Ova-specific T cells within transplanted pLNs and mLNs (Fig. 1b). Strikingly, the high Treg-inducing capacity of mLNs was maintained fifty weeks after transplantation into the popliteal fossa (Fig. 1b-c), demonstrating that mLN SCs can stably and durably maintain their tolerogenic properties in a skin-draining environment.

Next, we investigated whether the tolerogenic properties of mLN SCs can resist inflammatory perturbations within the intestine. First, mice were orally infected with enteropathogenic *Yersinia pseudotuberculosis*, a gastrointestinal pathogen known to enter mLNs shortly after infection (Supplementary Fig. 1A). As expected, *Yersinia* infection resulted in profound changes of the mLN SC compartment. At day 3 post infection (p.i.), the number of CD45−CD24−gp38−CD31− FSCs was significantly reduced compared to uninfected controls, and FSCs displayed an activated phenotype with increased MHCII expression (Supplementary Fig. 1B-C). Four weeks p.i., a time point when *Yersinia* were cleared from mLNs (Supplementary Fig. 1A), the number of FSCs was significantly increased, and the FSCs still showed an activated phenotype (Supplementary Fig. 1B-C), suggesting that the FSCs had significantly proliferated in response to the infection. To assess whether infection-induced changes to the mLN SC compartment can persistently alter the high Treg-inducing capacity of mLNs, we transplanted mLNs of mice four weeks p.i. with *Yersinia* into the popliteal fossa of uninfected recipient mice. Eight to ten weeks later the Treg-inducing capacity of transplanted mLNs was analyzed as described above, so that any impact of previous infection on the frequency of de novo induced Foxp3+ Tregs could be observed (Supplementary Fig. 1D). This analysis indicated that the observed infection-induced changes to the mLN SC compartment did not persistently alter the high Treg-inducing capacity of mLNs. In a second approach, we utilized the chronic dextran sodium sulfate (DSS) colitis model to study whether a chronic inflammatory perturbation could abrogate the high Treg-inducing properties of mLN SCs. After four cycles of DSS treatment (Fig. 1d), when mice had developed a chronic colitis as indicated by a significant shortening of colon length, as well as increased spleen size (Fig. 1e), mLNs and LNs draining the caecum and proximal colon (caeLNs) were transplanted into the popliteal fossa of recipient mice as described above. Interestingly, eight to ten weeks after transplantation, both caelNs and mLNs still showed a high Treg-inducing capacity (Fig. 1f). Together, these results highlight the stability of the tolerogenic properties of mLN SCs, by withstanding acute and even chronic inflammatory perturbations.
Microbiota imprint tolerogenic properties into mLN SCs. Our previous findings indicated that microbiota might contribute to the durable imprinting of tolerogenic properties in mLN SCs, as transplanted mLNs taken from germ-free (GF) mice did not show any increased Treg-inducing capacity. To further underpin the role of microbiota in this imprinting process, GF mice aged three days were simultaneously colonized by specific pathogen-free (SPF) mice. Subsequently, mLNs from co-housed GF mice were transplanted into the popliteal fossa of SPF-housed recipient mice. Eight to twelve weeks after transplantation, the Treg-inducing capacity of transplanted LNs was analyzed as described before. Interestingly, when we determined the frequency of de novo induced Foxp3+ Tregs, no difference between transplanted mLNs taken from co-housed GF mice and transplanted mLNs of age-matched SPF controls (Fig. 2b) was observed. These data indicate that microbiota are sufficient to stably imprint tolerogenic properties within mLN SCs.

Intact mLN SC tolerogenic property after microbiota changes. Having confirmed that microbiota can permanently shape the immunomodulatory capacity of mLN SCs, we next asked whether global alteration of the microbiota composition during the critical neonatal period might influence this imprinting process. Therefore, we treated pregnant mothers starting from embryonic day 7 and their offspring with various antibiotics, namely streptomycin, polymyxin B and vancomycin, all targeting different classes of bacteria (Fig. 2c). As expected, these treatments led to alterations of the microbiota composition, with vancomycin causing the
most drastic changes and resulting in an elevated proportion of Lactobacillaceae as Gram-positive Lactobacillus spp. are commonly vancomycin-resistant (Supplementary Fig. 2C–D). Next, mLNs of antibiotic-treated offspring were transplanted into the popliteal fossa of untreated recipient mice, followed by assessment of their Treg-inducing capacities as described before. None of the treatment regimens abrogated the tolerogenic properties of mLN SCs. Remarkably, vancomycin, which caused the strongest reduction in microbiota diversity, actually resulted in enhanced Treg induction within transplanted mLNs (Fig. 2c, Supplementary Fig. 2E).

Since our data indicated that different microbiota compositions are capable of imprinting tolerogenic properties within mLN SCs, albeit to different degrees, we next inquired which bacteria...
derived molecular signals were required for imprinting. Intestinal microbiota provide different metabolites, and particularly short-chain fatty acids (SCFA) and aryl-hydrocarbon receptor (AhR) ligands which have been shown to contribute to lasting alterations of immune homeostasis\(^1\)\(^{-3}\)\(^,\)\(^4\)\(^,\)\(^2\)\(^2\). Thus, we here assessed whether these ligands are sufficient to imprint high Treg-inducing capacity into mLN SCs. GF mice were orally treated for four weeks with either propionate, butyrate or a mixture of endogenous AhR ligands, namely 2-((1H-indole-3-ylcarbonyl)-4-thiazolecarboxylic acid methyl ester (ITE), indole-3-acetic acid (IAA) and indole-3-carboxaldehyde (ISC). mLNs of treated GF mice were transplanted into the popliteal fossa of untreated SPF mice, followed by assessment of their Treg-inducing capacities as described before. Neither SCFAs nor AhR ligands were sufficient to imprint tolerogenic properties into mLN SCs (Fig. 2d). In summary, our findings indicate that microbiota can imprint tolerogenic properties within mLN SCs independent of their composition and major immunomodulatory metabolites.

**Microbiota imprint tissue-specific properties into mLN FSCs.** Next, we aimed to unravel the impact of microbiota on mLN SCs at the molecular level. Previous studies provided comparative microarray analyses of FSCs isolated from pLNs and mLNs revealing differential expression of several genes\(^2\)\(^3\)\(^,\)\(^4\)\(^,\)\(^2\)\(^4\) However, these studies did not investigate if microbiota influence the LN-specific transcriptional signatures of FSCs. Thus, we isolated FSCs from mLNs and pLNs of both SPF and GF mice. FSCs were subjected to transcriptome analyses by RNA-seq. Surprisingly, global inspection of these data by hierarchical clustering of differentially expressed genes (DEGs, \(\log_2(\text{FC})\) \(\geq 1\), \(q\)-value \(\leq 0.05\)) revealed that colonization status scarcely impacted the transcriptomes of FSCs from pLNs and mLNs alike (Fig. 3a). A direct comparison of all four conditions underlined the impact of anatomic localization on the transcriptional signatures of FSCs (Supplementary Fig. 3A). Gene ontology (GO) analysis revealed that key biological processes including RA metabolism were maintained independent of microbial colonization (Supplementary Fig. 3B, Supplementary Table 1).

Having demonstrated that colonization status barely affects the transcriptome of FSCs from mLNs, we next sought to unravel whether microbiota could be instrumental in stabilizing the mLN-specific transcriptional signature. Accordingly, we transplanted mLN-SPF or mLN-GF to the skin-draining popliteal fossa of SPF recipient mice, while pLN-SPF were transplanted as a surgical control. Eight to fifteen weeks later, FSCs were isolated from transplanted LNs and subjected to low-input RNA-seq (RNA-seq\(^\text{\textsuperscript{1}}\)), with FSCs isolated from endogenous LNs as additional controls. Interestingly, FSCs from transplanted mLN-SPF maintained a substantial fraction of the mLN-specific transcriptional signature, with 108 and 368 genes showing persistent up-regulation and down-regulation, respectively (Fig. 3b left, Supplementary Table 2). In contrast, FSCs from transplanted mLN-GF were strongly influenced by the skin microenvironment and almost completely adapted to the transcriptional signature of FSCs from pLNs (Fig. 3b right). With 32 up-regulated and 49 down-regulated genes only a small fraction of the mLN-specific transcriptional signature was maintained (Fig. 3b right, Supplementary Fig. 3C). Together, these data suggest that microbiota are essential for stabilizing the mLN-specific transcriptional signature within FSCs.

GO analysis of those DEGs maintained in FSCs from transplanted mLN-SPF revealed a permanent repression of inflammation-associated biological pathways, while RA biosynthesis remained stably up-regulated (Fig. 3c). Remarkably, Aldh1a2, an enzyme responsible for RA synthesis, was persistently

![Image](file_url)

**Fig. 3** Location-specific transcriptional signatures of mLN FSCs are imprinted by microbiota. a CD45<sup>-</sup> Ter119<sup>-</sup> CD31<sup>-</sup> gp38<sup>+</sup> FSCs were isolated from mLNs and pLNs of GF or SPF mice, and RNA-seq and subsequent analysis was performed. DEGs were identified in colonization-dependent (SPF vs. GF) and location-dependent (mLNs vs. pLNs) pairwise comparisons (\(\log_2(\text{FC})\) \(\geq 1\) and \(q\)-value \(\leq 0.05\)). Heatmap represents 1411 DEGs. Mean-centered \(\log_2(\text{RPKM})\) values are depicted. Data pooled from two independent experiments. b CD45<sup>-</sup> CD24<sup>-</sup> CD31<sup>-</sup> gp38<sup>+</sup> FSCs were isolated from endogenous mLN-SPF and pLN-SPF, transplanted pLN-SPF and mLN-SPF or transplanted mLN-GF. RNA-seq\(^\text{\textsuperscript{\text{1}}}\) and subsequent analysis was performed. Colored numbers in scatterplots represent DEGs (\(\log_2(\text{FC})\) \(\geq 1\) and \(q\)-value \(\leq 0.05\)) for the pair-wise comparisons of FSCs. On the x-axis, log(FC) of gene expression from FSCs (endogenous LNs) is plotted. (Left) On the y-axis \(\log_2(\text{FC})\) of gene expression from FSCs from transplanted mLN-SPF vs. pLN-SPF is plotted. (Right) On the y-axis \(\log_2(\text{FC})\) of gene expression from FSCs from transplanted mLN-GF vs. pLN-SPF is plotted. Data pooled from two to four independent experiments (n = 3–8). c GO analysis of biological processes of genes persistently up-regulated (Maintained, blue dot) or down-regulated (Represse, pink dot) in FSCs of transplanted mLN-SPF. d Heatmaps of expression of genes involved in RA metabolic process or of soluble mediators within FSCs from indicated groups. Numbers in brackets indicate average \(\log_2(\text{RPKM})\) expression of respective genes across all experimental groups. Data pooled from two to four independent experiments (n = 3–8). DEG differentially expressed genes, AA amino acid, FC fold-change, FSC fibroblast stromal cells, GO gene ontology, RA retinoic acid, reg. regulation, res. response, RPKM reads per kilobase of exon length per million mapped reads, sig. signaling.
maintained only in FSCs from transplanted mLN-SPF but not in FSCs from transplanted mLN-GF, while the RA-degrading enzyme Cyp26b1 remained repressed (Fig. 3d). Several important soluble mediators (Tnfsf18, Cxcl12, Cxcl16, Il7, Ccl19, Ccl21a) were maintained at lower levels in FSCs from transplanted mLN-SPF, but showed increased expression levels in FSCs from transplanted mLN-GF (Fig. 3d). In summary, transcriptome analysis of FSCs isolated from transplanted mLNs confirmed that the stabilization of a substantial fraction of the location-specific transcriptional signature depends on microbiota.
Location-dependent features are imprinted within SC subsets. The complex anatomical infrastructure of the LN requires a separation of functional properties within SC subsets. However, the precise role of FSC subsets cannot be resolved by transcriptional profiling of bulk FSCs. To get a fair picture of the SC subset composition within pLNs and mLNs of adult SPF mice and gain preliminary insights into the functional properties of these subsets, we performed single-cell RNA-seq (scRNA-seq) of the entire CD24−CD45− cell population and acquired the transcriptomes of quality-controlled 3928 and 5329 single-cells for mLNs and pLNs, respectively. For integrative analysis of scRNA-seq of mLNs and pLNs we performed sample alignment based on diagonal canonical correlation analysis to overlay the transcriptomes of both LNs and performed unsupervised clustering using t-SNE (Supplementary Fig. 4A). At first glance, we recognized known major LN SC subpopulations, including lymphatic endothelial cells (LEC, Pdpn+Pcam1+), blood endothelial cells (BEC, Pdpn−Pcam1+) and non-endothelial SCs (SC, Pcam1−Ackr4+), with the latter displaying the most pronounced heterogeneity. Importantly, Pdpn and Pcam1 expression alone were insufficient to separate LECs, BECs and non-endothelial SC at a single-cell level, although sufficient to distinguish cellular clusters based on the averaged expression (Supplementary Fig. 4A).

To get an unbiased picture of SC subsets within pLNs and mLNs, we aligned 2786 mLN SCs and the identical number of randomly sampled pLN SCs, while omitting all LECs and BECs (Fig. 4a). Fourteen transcriptional clusters harboring unique functional profiles were identified based on DEGs and GO analysis (Fig. 4a–b, Supplementary Fig. 4A–C). Importantly, the vast majority of clusters were found in both pLNs and mLNs (Supplementary Fig. 4D), suggesting that LNs are composed of similar SC subsets. We could recapitulate known SC subsets based on gene expression patterns, namely Acta2+ pericytes (PvC) and Ccl19+ T cell zone reticular cells (TRC) (Fig. 4a–b). Among the TRCs we were able to distinguish four subsets, namely Cxcl9+, Cc11rhigh, Ccl19+ and Ccl19+Ptgishigh TRCs (Fig. 4a–c). Additionally, a subset expressing a variety of inflammatory and chemotactic mediators including Il6, Cxcl1, Ccl2, Ccl7, and Cxcl2, here termed Il6+Cxcl1+ SCs, was identified (Fig. 4a–c, Supplementary Table 3). We also detected subsets with higher, but not exclusive expression for Inmnt and Nr4a1, previously termed Inmnt+ and Nr4a1+ SCs, respectively. Remarkably, a large population of CD34+ SCs, known to predominantly locate at the LN capsule and around larger vessels, was identified that could further be subdivided into four distinct subsets, namely CD34+(Aldh1a2+), CD34+(Ackr3+), CD34+(Cd10+), and CD34+(Cd28+) SCs, each bearing distinct transcriptional profiles (Fig. 4a–c, Supplementary Fig. 4C, Supplementary Table 3). Finally, two subsets enriched for proliferating (pSC) and metabolically active (mSC) were detected (Fig. 4a, Supplementary Fig. 4c, Supplementary Table 3). By projecting transcriptional signatures from mLN SC clusters to pLN SC clusters using scmap, the majority of SC subsets were reflected (Supplementary Fig. 4D). This finding is underscored by the overlap of the top 10 DEGs per cluster (Supplementary Fig. 4E, Supplementary Table 4 and 5), demonstrating a substantial conservation of LN SC subsets across different LNs.

Having identified novel non-endothelial SC subsets, we next asked if any one of these subsets could mainly account for the stably imprinted, mLN-specific transcriptional signature discovered within the total population of FSCs isolated from transplanted mLN-SPF (Fig. 3b–d, Supplementary Table 2). Thus, we calculated the cumulative Z-score (cZscore) for the 108 persistently up-regulated and 368 persistently down-regulated genes across all newly identified SC clusters identified by scRNA-seq (Fig. 4d). Interestingly, defined sets of the persistently up-regulated genes were mainly expressed by the four CD34+ SC subsets, as indicated by a cZscore > 0, but also in Cxcl9+ TRCs and PvCs (Fig. 4d). A similar picture was observed for the persistently down-regulated genes (cZscore < 0) (Supplementary Fig. 4f).

In order to identify the location of Aldh1a2-expressing non-endothelial SCs, we performed immunostaining and confocal imaging on tissue sections of mLN-SPF and pLN-SPF from chimeric mice, which were generated by reconstituting Ccl19CreGroxRosa2614td individuals with bone marrow from Zbb46GFP reporter mice, allowing us to differentiate Ccl19-dependently fate-mapped SCs and Zbb46-expressing DCs (Supplementary Fig. 5A–C). As expected, Aldh1a1/2+ DCs were abundantly found in mLNs but not pLNs, whereas no Aldh1a1/2-expressing SCs could be detected within the T cell zone (Supplementary Fig. 5B). It should be noted that a reliable identification of Aldh1a2-expressing SCs within the capsular region and in the vicinity of the medullary cords was prevented by unspecific background signals. In summary, the scRNA-seq data suggest that selected immunomodulatory properties of mLN SCs are confined to distinct SC subsets, preferentially of the CD34+ SC group.

mLN SCs modulate resident DC composition and transcriptome. Emerging evidence suggests that LN SCs and DCs closely interact. Due to the location of FSCs within the T cell zone of the LN, and the previously reported superior Treg-inducing capacity of DCs from mLNs, we here aimed to determine the direct modulation of DCs by LN SCs. Thus, we transplanted mLNs and pLNs from adult SPF mice to the skin-draining popliteal fossa of SPF recipient mice. Eight to sixteen weeks later, we first assessed the composition of CD11c+MHCII+ resident DC (resDC) and CD11c+MHCIIhi migratory DC (migDC) subsets in transplanted LNs (Fig. 5a). It is important to note that in the LN transplant setting all resDCs and migDCs of donor origin have been replaced by recipient-derived DCs at the
Fig. 5 mLN SCs modulate subset composition and transcriptional signature of resident DCs. Indicated LNs were transplanted to the popliteal fossa of SPF-housed mice. Eight to sixteen weeks later, single cell suspensions were generated by enzymatic digestion. a-d Single cell suspensions were analyzed using flow cytometry. a Exemplary dotplot of viable Lin<sup>−</sup> cells from transplanted LN. (b) Exemplary dotplot of subsets from resDCs and migDCs from indicated LNs. c-d Scatterplot shows frequencies of indicated subsets among migDCs (c) and resDCs (d); data pooled from two to three independent experiments (n = 12-16). e resDCs were isolated from indicated LNs. RNA-seq<sup>1</sup> and subsequent analysis was performed. Colored numbers in scatterplots represent DEGs for the respective pair-wise comparisons of resDCs (|log2(FC)| ≥ 1 and q-value ≤ 0.05). On the x-axis log2FC of gene expression from resDCs (endogenous LNs) is plotted. On the y-axis log2(FC) of gene expression from resDCs of transplanted mLN-SPF vs. pLN-SPF is plotted. f Heatmap of expression of genes persistently down- (pink dot, left) or up-regulated (blue dot, right) in resDCs isolated from transplanted mLN-SPF. Numbers in brackets indicate average log2(RPKM) expression of respective genes across all experimental groups. Data pooled from two to three independent experiments (n = 3-8). migDC migratory dendritic cell (Lin<sup>−</sup>CD11c<sup>+</sup>MHCII<sup>high</sup>), resDC resident dendritic cell (Lin<sup>−</sup>CD11c<sup>high</sup>MHCII<sup>+</sup>), Lin CD3<sup>+</sup>CD45R<sup>+</sup>Ly6G<sup>−</sup>F4/80<sup>high</sup>, FC fold-change, RPKM reads per kilobase of exon length per million mapped reads, Endo endogenous, Tx transplanted.
resDCs from transplanted mLN s retain Treg-inducing capacity. Finally, we wanted to assess the Treg-inducing capacity of resDCs and migDCs shaped by mLN SCs. First, we ruled out any direct impact of mLN SCs on naïve T cells during de novo Treg induction. In vitro co-cultivation of polyclonally activated naïve T cells with CD24+CD45+CD31+ FSCs isolated from mLNs was unaccompanied by an increased frequency of de novo induced Foxp3+ Tregs when compared to co-cultures with FSCs isolated from pLNs (Supplementary Fig. 7A). The lack of any direct impact of FSCs on Treg induction was further corroborated in the context of TGFβ1-dependent Treg induction in conjunction with blocking Timd4, a surface molecule predominantly expressed by FSCs and described to inhibit Treg induction42, further suggesting that DCs are required to confer FSC-dependent immune modulation (Supplementary Fig. 7A).

Hence, we isolated resDCs and migDCs from transplanted LNs eight to sixteen weeks after transplantation, while resDCs, as well as migDCs isolated from endogenous LNs were taken as controls. All DCs were cultured with Ova-TCR transgenic naïve T cells in the presence of Ova peptide, and the frequency of de novo induced Foxp3+ Tregs was analyzed on day 5. In all conditions tested, a comparable proliferation was observed (Fig. 6a). The high Treg-inducing capacity of migDCs from endogenous mLNs was not maintained in migDCs isolated from transplanted mLNs (Fig. 6a–b). In contrast, resDCs from transplanted mLNs fully attained their higher Treg-inducing capacity (Fig. 6a and c). Interestingly, GO analysis of the mLN SC-dependent modulation of resDCs pointed towards genes involved in bone mineralization, including up-regulation of Bmp2 encoding the bone morphogenic protein 2, whereas Tgfβ1 and Tgfβ3 were expressed at equal levels in resDCs from pLNs and mLNs (Fig. 6d, e). Bmp2 belongs to the TGF-β superfamily and promotes TGFβ1-dependent Treg induction solely in a synergistic manner. When culturing resDCs from mLNs and pLNs in the presence of the Bmp-antagonist Noggin, mLN resDC-mediated Treg induction was significantly reduced (Fig. 6f), whereas exogenous supplementation with Bmp2 resulted in an increased frequency of de novo
induced Foxp3+ Tregs in the presence of mLN resDCs (Supplementary Fig. 7B). Together, these results suggest that mLN SCs can directly modulate resDCs and instruct them with Treg-inducing properties, thereby substantially contributing to the maintenance of intestinal tolerance.

**Discussion**

Microbiota contribute to the acquisition of unique properties within the intestinal immune system, including the imprinting of a high Treg-inducing capacity in mLN s11. Here, we identify a critical time period for this imprinting process, delineate which non-endothelial SC subsets compile imprinted functional properties, and provide evidence that mLN SCs can shape functional properties of resDCs to attain a high Treg-inducing capacity.

Building on our previous observation that mLN SCs display stable tolerogenic properties11, we here challenged mLN SCs concerning their tolerogenic function by exciting infectious and inflammatory perturbations. Infecting mice with Y. pseudotuberculosis resulted in the strong activation and proliferation of FSCs, in line with previous studies21,24. Remarkably, the tolerogenic properties of mLN SCs were not affected by this infectious perturbation, even though Y. pseudotuberculosis infection had been reported to propagate long-lasting, microbiota-dependent inflammation of mesenteric adipose tissue, finally resulting in an abrogation of mLN-dependent oral tolerance44. Moreover, chronic intestinal inflammation induced by repetitive DSS treatment failed to abrogate the high Treg-inducing capacity of mLN s, consistent with a recent report demonstrating a high topological robustness of the FSC network45. Together, these findings imply that the microbiota-dependent imprinting of tolerogenic properties within mLN SCs provides a very stable microenvironmental framework for efficient de novo Treg induction resistant to strong inflammatory perturbations.

Importantly, we identified the neonatal phase as the critical time period for the microbiota-dependent imprinting of the high Treg-inducing capacity within mLN SCs. During this phase, early after birth, the intestinal mucosa is rapidly colonized with microbiota, which has a profound impact on the development and maturation of a fully functional mucosal and systemic immune system2–4. Interference with the microbiota composition by antibiotic treatment revealed that a low-diversity microbiota was sufficient to imprint tolerogenic properties within mLN SCs. Remarkably, microbiota with an elevated proportion of Lactobacillaceae, prominent early colonizers of the intestine46, fostered the imprinting of an even higher Treg-inducing capacity within mLN SCs, implicating evolutionarily conserved mechanisms to instill tolerance at the priming site of intestinal immune responses. It is important to note that the microbiota-dependent imprinting of tolerogenic properties within mLN SCs is not merely restricted to the neonatal phase as colonization of adult GF mice also resulted in stable Treg-inducing properties within mLN s, implying that mLN SCs retain functional flexibility until encountering microbiota-derived signals. These findings contrast with other microbiota-dependent tolerogenic mechanisms known to operate solely during the neonatal phase but not in adults1,3,4.

Surprisingly, microbiota-derived signals scarcely impacted the transcriptomes of FSCs. In accordance with previous comparative microarray analyses23,24, a large number of DEGs were identified between FSCs from pLN s and mLN s. Together, these data suggest that anatomic localization mainly determines the transcriptomes of FSCs rather than colonization status. Nonetheless, microbiota-derived signals were essentially required to stabilize the unique mLN-specific transcriptional signature within FSCs as evidenced by our finding that FSCs from transplanted mLN-GF were strongly influenced by the skin microenvironment and almost completely adapted to the transcriptional signature of FSCs from pLN s, while FSCs from transplanted mLN-SPF maintained a part of the mLN-specific transcriptional signature. The power of scRNA-seq47 allowed attribution of these persistently up-regulated, as well as down-regulated genes to selected FSC subsets. Importantly, only a few of the fourteen transcriptional clusters identified here were known before15, and a number of FSC subsets harboring unique immunomodulatory profiles are described here for the first time.

It is widely accepted that FSCs of secondary lymphoid organs regulate lymphocyte compartmentalization through chemokine secretion, form distinct niches for interaction with hematopoietic cells, and participate in the orchestration of appropriate cell-cell interactions required for adaptive immunity11. Splenic SCs can drive DC development from hematopoietic progenitors48, maintain DC homeostasis49, and support the differentiation of mature DCs to attain a tolerogenic phenotype50. Recently, FSCs from mLN s were shown to produce IL-15 for the maintenance of group 1 innate lymphoid cells51, and that FSCs from LN s can function as niche cells, supporting the Notch-mediated differentiation of DCs, as well as follicular helper T cells52. In the present study we demonstrate that mLN SCs, once imprinted by microbiota, can directly modulate resDCs and instruct them with Treg-inducing properties. DCs are essential cellular players during the peripheral de novo generation of Foxp3+ Tregs11. Previous studies have demonstrated that within the lamina propria migDCs can acquire the unique capacity to produce RA via direct crosstalk with lamina propria SCs in a RA- and GM-CSF-dependent manner53, suggesting the functional education of migDCs within the intestinal tissue54 which leads to the high Treg-inducing properties of CD103+ migDCs within mLN s10,55. Interestingly, in the LN transplant setting we observed that skin-derived migDCs do not attain Treg-inducing properties when entering the transplanted mLN-SPF, suggesting that mLN SCs are incapable of reprogramming the functional properties of skin-derived migDCs. In contrast, resDCs could be efficiently modulated by SCs from transplanted mLN-SPF, as evidenced by subset composition, transcriptional signature and Treg-inducing properties. While the molecular mechanisms underlying this modulation still remain enigmatic, potential candidate genes could be deduced from the mLN-specific transcriptional signature stably maintained within FSCs from transplanted mLN-SPF. The RA-synthesizing enzymes Aldh1a2 and Aldh1a3, showing a mutually exclusive, persistent expression within two distinct subsets of the adventitial/capsular CD34+ SCs28, are prime candidates as RA had been shown to modulate functional properties of DCs54. Although we were able to confirm that Aldh1a2/3 expression is higher in DCs from mLN s as compared to pLN s, defining the precise localization of the adventitial CD34+ SCs expressing Aldh1a2/3 proved elusive, due to the unspecific binding of the applied detection antibody and the higher autofluorescence in the medullary areas of the LN. Other methods such as NICHE-seq or mRNA-based microscopy would thus be more appropriate56,57.

When analyzing the transcriptional signature of resDCs that had been modulated by mLN SCs, several genes showing persistent up-regulation were identified. Among these Bmp2 was shown to act in concert with TGF-β in inducing Tregs43. As blocking of Bmp2-mediated signaling via Noggin abrogated mLN resDC-dependent Treg induction, mLN SC-dependent DC modulation could contribute to the maintenance of the tolerogenic properties of resDCs within mLN s, by elevating the Treg induction potential of resDC instilling higher Bmp2 expression.

In summary, we showed that microbiota stably and durably imprint tolerogenic properties within mLN SCs in the neonatal phase, rendering these resistant to inflammatory perturbations.
In the mouse strain, Foxp3^{GFP} x Rag2^{−/−} x DOI11.10 (BALB/c), Foxp3^{GFP} x Rag2^{−/−} x DOI11.10 (BALB/c), CD45.1 (C57BL/6 and BALB/c), CD45.2 (BALB/c), CD45.1 (C57BL/6), Anti-CD69 (C57BL/6), APC-Cy7 (C57BL/6), and BALB/c mice from Janvier were bred and were kept under SPF conditions in isolated veterinary cages at the Helmholtz Centre for Infection Research (Braunschweig, Germany). GM mice (BALB/c) were generated at Hannover Medical School (Hannover, Germany) by cesarean section and maintained either in plastic film isolators or in static micro-isolators (gnotocages) at Hannover Medical School or the Helmholtz Centre for Infection Research (Braunschweig, Germany). If not stated otherwise, water and Ova-free diet were supplied ad libitum. In all experiments, gender- and age-matched mice were used. All mice were housed and handled in accordance with good animal practice as defined by the German national animal welfare bodies. All animal experiments were approved by the local animal ethic committee on the Ethics of Animal Experiments, as well as the responsible state office (Lower Saxony State Office for Consumer Protection and Food Safety) under the permit number 33.4-2.502.04-12.012. Cells were purchased from EMMA (INFRAFRONTIER), Roes266731 and ZippyBlue at 1:800 eBioscience Cat. 103212; PercP-Cy5.5 at 1:800 BioLegend Cat. 103236), anti-CD49b (clone A20; BUV395 at 1:200 BD Cat. 565212), anti-CD45.2 (clone 104; PE-Cy7 at 1:400 eBioscience Cat. 25-0311-82), anti-CD45 (clone 30-F11; FITC at 1:2000 BioLegend Cat. 115512), anti-CD24 (clone M1/69; FITC at 1:1000 eBioscience Cat. 17-0032-82; PerCP-Cy5.5 at 1:400 BioLegend Cat. 300214; PE-Cy7 at 1:400 BioLegend Cat. 309214; PerCP-Cy5.5 at 1:400 BioLegend Cat. 100218), anti-CD19 (clone 6D5; APC at 1:400 BioLegend Cat. 118516), anti-CD19 (clone 6D5; APC at 1:400 BioLegend Cat. 118516). Fluorochrome-conjugated streptavidin and anti-mouse CD16/CD32 antibody (BioXcell) for 5 min on ice. Live/dead discrimination was carried out utilizing LIVE/DEAD Fixable Dead Cell Stain (Invitrogen) according to the manufacturer’s recommendations. Surface staining was performed for 15 min on ice in PBS containing 0.2% BSA. Cells were washed, resuspended in PBS containing 0.2% BSA and measured at 530 nm using a Fortessa flow cytometer with FlowJo software (Tree Star). LN transplantsations. For transplantsations into the popliteal fossa, BALB/c-recep- tive or C57BL/6-receptive mice were anesthetized with ketamine (WDT) and xylazine (CP Pharma), the skin of the popliteal fossa of the right hind leg opened, and the subcutaneous pLN and fat tissue removed. PBS or mLNs dissected from donor mice were placed into the popliteal fossa, and the cut sewn with absorbable suture (Catgut). Before subjecting to further experimental procedure, recipients were housed for a minimum of eight weeks to ensure restoration of the lymphatic and blood vessel system. For all experiments, successful engraftment of transplanted LNs into the popliteal fossa was assessed by footpad injection of 20 µl Patent V (25 mg/ml Sigma-Aldrich) into CO2-anesthetized mice. T-cell isolation and adoptive transfer. For adoptive transfer of cells from Foxp3^{GFP} x Rag2^{−/−} x DOI11.10 mice, single cell suspensions were generated from spleens and LNs and transferred without cell sorting. Before transfer, cells were labeled with cell proliferation dye CPDviolet (Invitrogen), and approximately 3 × 10^5 cells injected in 100 µl PBS (ThermoFisher Scientific) i.v. per recipient mouse. Immunization. For de novo Foxp3 Treg induction, 20 µg Ova257-264 peptide was injected i.v. on two consecutive days, starting one day after adoptive transfer of cells from Foxp3^{GFP} x Rag2^{−/−} x DOI11.10 mice. At indicated time points after immunization, cells were isolated from transplanted and endogenous LNs. Gastrointestinal infection. Prior to infection with *Yersinia pseudotuberculosis* strain YPIII (Janvier, France), food was removed for 12 h. Subsequently, BALB/c mice were injected intragastrically with 10^9 bacteria (in 200 µl PBS) that had been cultivated for 12 h at 25 °C in LB medium (BD). At indicated time points p.i., the bacterial load within mLNs was determined using single cell suspension aliquots homo- geneously mixed at 30,000 rpm for 30 s using a Polytron PT 2000 homogenizer (Kinematica) and serial dilutions of the homogenates plated on LB plates. Colony forming units (CFU) were counted and are given as CFU/organ. For transplantation, separate cohorts of mice were utilized at the indicated time points p.i. Chronic DSS colitis. To induce a chronic DSS colitis, mice were treated for four days with 5% DSS (36–50 kDa, MP Biomedicals) in drinking water ad libitum for four days followed by ten days of normal drinking water. Changes in body weight were monitored over time. At the end point of the experiment, colon length and spleen weight were determined. Antibiotic treatment. Antibiotics were added to the drinking water seven days post conception with vancomycin hydrochloride at 200 mg/l streptomycin sulfate at 200 µg/l or polymyxin B sulfate at 100 µg/l (all Sigma-Aldrich). Antibiotic solutions were exchanged twice per week. Offspring continuously received antibiotic treatment until donation of mLNs at age of four to five weeks. Bone marrow chimeras. Mice were y-irradiated (7 Gy) from an X-Ray source and reconstituted with at least 5 × 10^6 total bone marrow cells from donor animals. Chimera individuals were kept at least 40 days prior to resection of mLNs and pLNs. Immunostaining and microscopy. LN sections were fixed in 4% formaldehyde for 30 min, blocked with PBS containing 1% BSA and 5% FCS for 1 h, and incubated with the indicated antibodies. Secondary antibodies were applied in PBS containing 1% BSA and 0.1% Tween-20 for 1 h. Sections were washed three times in PBS. Immunofluorescence confocal microscopy was performed using a Zeiss LSM 880 confocal microscope. Separate images were collected for each fluorochrome and overlaid to obtain a multicolor image. Final image processing was performed with Imaris software (Bitplane). 16 S rDNA sequencing. DNA extraction, amplification of the V1-2 region of the 16 S rDNA gene and bioinformatics processing were performed as previously described with minor modifications. The initial PCR was performed for 20 cycles with the following primers: 27 F: 5′-AGAGTTTGATCMTGGCTCAG-3′; 319 R: 5′-GTATTACCTTCTTACACGAGCTCTTCGATTTATGACCAAGATTGTTTACCGTCGTACGCAG-3′. Barcodes, indexes and Illumina sequence adaptors were appended via two additional PCR reactions. Firstly, a barcode was added in 15-cycle PCR reaction (Exemplary barcode indicated in italic). In a second step, an additional PCR was performed for 20 cycles with the following primers: 27 F: 5′-ACACTCTTTCCCTACACGACGCTCTTCCGATCT-3′; 319 R: 5′-CAAGAGTTTGATCMTGGCTCAG-3′. Initial PCR was followed by a second, dilution PCR to ensure that the barcode was visible. Following purification, PCR products were amplified in two steps. DNA concentration and quality were measured using Qubit dsDNA HS Assay Kit (ThermoFischer) and a Qubit fluorometer. DNA sequencing was performed at the company's sequencing service using the Illumina HiSeq X Ten platform. FASTQ files were obtained from this sequencing run. The Q20 score was above 98% in all samples. The next step was to demultiplex the FASTQ files using the FastqDemux tool (Micron Microtech) for 2h, washed in 0.1 M phosphate buffer, and dehydrated overnight in 30% sucrose at 0.1 M phosphate buffer. Tissues were snap frozen and 35 µm frozen sections stained with the indicated antibodies in 0.1 M Ultrapure Tris Buffer (ThermoFisher) containing 0.5% w/v BSA and 1% Triton X-100. Immunofluorescence confocal microscopy was performed using a Zeiss LSM 880 confocal microscope. Separate images were collected for each fluorochrome and overlaid to obtain a multicolor image. Final image processing was performed with Imaris software (Bitplane).
In vitro Treg-induction assay-SCs

For the co-culture of DC subsets and naive CD4+ T cells, single cell suspensions were generated from spleens and LNs of Foxp3ε+Rag2−/−xDOI1.10 mice, followed by enrichment of CD4+ T cells using CD4 (L3T4) MicroBeads and autoMACS separation. CD4+ T cells were then labeled with cell proliferation dye CPDviolet. At day 5, cells were resuspended, labeled with fluorochrome-conjugated antibodies, and the frequency of Foxp3εCD2D+ cells among CD4+ T cells determined by flow cytometry.

DC isolation.

To isolate DC subsets, transplanted or endogenous pLNs and mLNs were excised and digested as described above. After digestion, cells were kept at 4°C in PBS containing 0.2% BSA and 5 mM EDTA (Roth). CD4+CD24+ cells were enriched by autoMACS separation after magnetic labeling of CD4+ cells using anti-CD4-APC (20-F11, eBioscience) followed by anti-APC microbeads (Millenyi Biotec) or anti-CD45 Antibeads (Biolegend). Subsequently, the CD4+ fraction was stained using fluorochrome-coupled antibodies and either analyzed directly by flow cytometry or used to sort CD4+CD24+CD103+ gd TCS (Aria III, 100 μm nozzle) and bulk CD4+CD24- non-hematopoietic cells (Aria III, 70 μm nozzle) for RNA-seq/RNA-seq2 and scRNA-seq, respectively.

Library preparation scRNA-seq.

Data were demultiplexed using Cell Ranger software (version 2.0.0) based on 8 bp 10X sample indexes and paired-end FASTQ files generated. The cell barcodes and transcript UMI counts were processed as previously described.68 Subsequently, read 2, which contains the transcript insert sequence, was aligned to mouse UCSC mm10 reference genome using STAR aligner with default parameters.69 The alignment results were used to quantify the expression level of mouse genes and generation of gene-barcode matrix, which were further processed with Seurat (version 2.3.1).68 The separately acquired mLNs and pLNs datasets were merged. All cells with less than 250 or more than 3,500 detected genes per cell were filtered out. Moreover, cells with more than 4.5% read mapping to mitochondrial genes were considered as dead cells and removed. The remaining cellular transcripts were normalized, log transformed and only cell clusters (non-endothelial SC) with expression for Pecam1 < 1 were used for the further analysis. Non-endothelial SC transcriptional signatures were regressed against the variables, number of UMI s and percent of mitochondrial reads. From the two independent experiments 2786 and 4483 non-endothelial SCs were obtained for mLNs and pLNs, respectively. Variable genes were identified with default settings. Based on the PCEI-bowPlot, 22 and 18 PCAs were used for the clustering of mLNs and pLNs, respectively. Clusters were defined at the resolution of 1.3 and 0.9 for mLNs and pLNs, respectively and DEGs identified with FindAllMarkers (Wilcoxon rank sum test, min.pct = 0.25, threshold = 0.25, only.pos = TRUE). Projection of transcriptional signatures from mLN SC clusters to pLN SC clusters was carried out using scmap with 1000 features and threshold set to 0.7. To perform diagonal canonical correlation analysis (CCA)69, mLNs and pLNs were additionally regressed against percent of ribosomal reads on a per cell basis, data sets were merged and the union of the variable genes was used. t-SNE dimensionality reduction was performed using the scran package and the union of the SM and the CCA and resolution set to 1.1. DEGs were identified as stated above. Gene ontology (GO) analysis was performed for differentially upregulated genes per cluster using TopGO. Cumulative Z-scores were calculated based on the scaled expression per gene per cluster across the defined gene signatures.

**Statistical analysis.**

Group sizes were estimated according to a presumed standard deviation (SD) and an expected type I error of <0.05. The sample size was adjusted, if required, based on initial results. For all figures, each data point represents a single mouse if not stated otherwise. In Treg induction assays, samples were excluded from the statistical analysis if <500 adaptively transferred OvaTCR+CD4+ cells could be acquired. For comparison of unmatched groups, two-tailed Mann-Whitney was applied. If comparing more than three groups one-way ANOVA followed by Bonferroni post-hoc correction was used. All data are presented as mean ± SD, and p-values <0.05 deemed significant (* p < 0.05; ** p < 0.01; *** p < 0.001; ns, not significant).
for all flow cytometry based data. A Life Sciences Reporting Summary for this paper is available online.

Data availability
The GEO accession number for all RNA-seq, RNA-seqX and scRNA-seq data reported in this paper is GSE16633

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References
1. Remz, H., Brandtzaeg, P. & Hornef, M. The impact of perinatal immune development on mucosal homeostasis and chronic inflammation. Nat. Rev. Immunol. 12, 9–23 (2012).
2. Backhde, F. et al. Dynamics and stabilization of the human gut microbiome during the first year of life. Cell. Host. Microbe 17, 690–703 (2015).
3. Zeissig, S. & Blumberg, R. S. Life at the beginning: perturbation of the intestinal adaptive immune landscape. Immunity 46, 532–548 (2017).
4. Armulik, A., Genove, G. & Betcholtz, C. Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. Dev. Cell 21, 193–215 (2011).
5. Hornef, M. W. & Fulde, M. Ontogeny of intestinal epithelial innate immune responses. Front. Immunol. 5, 474 (2014).
6. Sakauchi, S., Miyara, M., Costantino, C. M. & Hafler, D. A. FOXP3+ regulatory T cells in the human immune system. Nat. Rev. Immunol. 10, 490–500 (2010).
7. Hsieh, C. S., Lee, H. M. & Liu, C. W. Selection of regulatory T cells in the thymus. Nat. Rev. Immunol. 12, 157–167 (2012).
8. Bilate, A. M. & Lafaille, J. J. Induced CD4+Foxp3+ regulatory T cells in immune tolerance. Annu. Rev. Immunol. 30, 733–758 (2012).
9. Cording, S. et al. The intestinal micro-environment imprints stromal cells to promote efficient Treg induction in gut-draining lymph nodes. Mucosal Immunol. 7, 359–368 (2014).
10. Hadi, U. et al. Intestinal tolerance requires gut homing and expansion of FoxP3+ regulatory T cells in the lamina propria. Immunity 34, 237–246 (2011).
11. Arpaia, N. et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature 504, 451–455 (2013).
12. Furuhashi, Y. et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature 504, 446–450 (2013).
13. Fletcher, A. L. et al. Reproducible isolation of lymph node stromal cells reveals site-dependent differences in fibroblastic reticular cells. Front. Immunol. 2, 35 (2011).
14. Malhotra, D. et al. Transcriptional profiling of stroma from inflamed and resting lymph nodes defines immunological hallmarks. Nat. Immunol. 13, 499–510 (2012).
15. Dobin, A. et al. STAR: ultrafast universal RNA-seq aligner. Bioinformatics 29, 15–21 (2013).
16. Armulik, A., Genove, G. & Betcholtz, C. Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. Dev. Cell 21, 193–215 (2011).
17. Rodda, L. B. et al. Single-cell RNA sequencing of lymph node stromal cells reveals niche-associated heterogeneity. Immunity 48, 1014–1028 (2018).
18. Sitnik, K. et al. Context-dependent development of lymphoid stroma from adult CD34+ adventitial progenitors. Cell Rep. 14, 2375–2388 (2016).
19. Kiselev, V. Y., Liu, A. & Hemberg, M. scmap: projection of single-cell RNA-seq data across data sets. Nat. Methods 15, 359–362 (2018).
20. Chai, Q. et al. Maturation of lymph node fibroblastic reticular cells from myofibroblastic precursors is critical for antiviral immunity. Immunity 38, 1013–1024 (2013).
21. Madisen, L. et al. A robust and high-throughput Cre reporting and characterization system for the whole mouse brain. Nat. Neurosci. 13, 133–140 (2010).
22. Satpathy, A. T. et al. Zbtb46 expression distinguishes classical dendritic cells and their committed progenitors from other immune lineages. J. Exp. Med. 209, 1135–1152 (2012).
23. Paasami, T., Pezoldt, J. & Henrich, J. Microenvironment matters: unique conditions within gut-draining lymph nodes favor efficient de novo induction of regulatory T cells. Proc. Mol. Biol. Transl. Sci. 136, 35–56 (2015).
24. Yeung, M. Y. et al. Interruption of dendritic cell-mediated TIM-4 signaling induces regulatory T cells and promotes cutaneous dendritic cell migration to lymph nodes during homeostasis and inflammation. Immunity 189, 3368–3377 (2012).
25. Uterm, M. H. et al. The atypical chemokine receptor CCR1L shapes functional CCL21 gradients in lymph nodes. Nat. Immunol. 15, 623–630 (2014).
26. Wilson, H. L., Ni, K. & O’Neill, L. A. NLRP3 inflammasome activation co-ordinated by the class B scavenger receptor Sirpalpha. Nature 540, 448–452 (2016).
27. Yeung, M. Y. et al. Interception of dendritic cell-mediated TIM-4 signaling induces regulatory T cells and promotes skin allograft survival. J. Immunol. 191, 4477–4485 (2015).
28. Lu, L. et al. Synergistic effect of TGF-beta superfamily members on the induction of Foxp3+ Treg. Eur. J. Immunol. 40, 142–152 (2010).
29. Brown, D. M. et al. Microbiota-dependent sequence of acute infection compromises tissue-specific immunity. Cell 163, 354–366 (2015).
30. Novakovic, M. et al. Topological small-world organization of the fibroblastic reticular cell network determines lymph node functionality. PLoS Biol. 14, e1002315 (2016).
31. Palmer, C., Bik, E. M., DiGiulio, D. B., Relman, D. A. & Brown, P. O. Development of the human infant intestinal microbiota. PLoS Biol. 5, e177 (2007).
32. Tanay, A. & Regev, A. Scaling single-cell genomicsto phenomenonology to mechanism. Nature 541, 331–338 (2017).
33. Wilton, H. L., Ni, K. & O’Neill, L. A. Identification of progenitor cells in long-term spleen stromal cultures that produce immature dendritic cells. Proc. Natl Acad. Sci. USA 97, 4784–4789 (2000).
34. Caton, M. L., Smith-Raska, M. R. & Reizis, B. Notch-RBPJ signaling controls the homeostasis of CD8+ dendritic cells in the spleen. J. Exp. Med. 204, 1653–1664 (2007).
35. Zhan, Z. et al. Splenic stroma drives mature dendritic cells to differentiate into regulatory dendritic cells. Nat. Immunol. 5, 1124–1133 (2004).
36. Gil-Cruz, C. et al. Fibroblastic reticular cells regulate intestinal inflammation via IL-15-mediated control of group 1 ILCs. Nat. Immunol. 17, 1388–1396 (2016).
37. Faustman, N. et al. Specific fibroblastic niches in secondary lymphoid organs orchestrate distinct Notch-regulated immune responses. J. Exp. Med. 211, 2265–2279 (2014).
38. Vicente-Suarez, I. et al. Unique lamina propria stromal cells imprint the functional phenotype of mucosal dendritic cells. Mucosal Immunol. 8, 141–151 (2015).
39. Mowat, A. M. & Agace, W. W. Regional specialization within the intestinal immune system. Nat. Rev. Immunol. 14, 667–685 (2014).
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Author contributions
J.P., M.P., M.Z., C.W., M.B., G.R.T., E.V., S.F., P.A., M.B., J.S., D.F., and M.V. performed experiments and interpreted data. D.-H.P., P.D., T.S., M.H., and U.B. provided expertise and feedback. M.B. and A.B. provided reagents. J.P., M.P., S.F., O.P., M.B., A.-E.S., and J. H. designed research, interpreted data, and wrote the manuscript.

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