Macrophage and dendritic cell subsets in IBD: ALDH<sup>+</sup> cells are reduced in colon tissue of patients with ulcerative colitis regardless of inflammation

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Disruption of the homeostatic balance of intestinal dendritic cells (DCs) and macrophages (MQs) may contribute to inflammatory bowel disease. We characterized DC and MQ populations, including their ability to produce retinoic acid, in clinical material encompassing Crohn’s ileitis, Crohn’s colitis and ulcerative colitis (UC) as well as mesenteric lymph nodes (MLNs) draining these sites. Increased CD14<sup>+</sup>DR<sup>int</sup> MQs characterized inflamed intestinal mucosa while total CD14<sup>+</sup> or CD1c<sup>+</sup> DCs numbers were unchanged. However, CD103<sup>+</sup> DCs, including CD141<sup>+</sup>CD103<sup>+</sup> and CD1c<sup>+</sup>CD103<sup>+</sup> DCs, were reduced in inflamed intestine. In MLNs, two CD14<sup>-</sup> DC populations were identified: CD11c<sup>+</sup>HLADR<sup>hi</sup> and CD11c<sup>+</sup>HLADR<sup>int</sup> cells. A marked increase of CD11c<sup>+</sup>HLADR<sup>int</sup> DC, particularly DR<sup>int</sup>CD1c<sup>+</sup> DCs, characterized MLNs draining inflamed intestine. The fraction of DC and MQ populations expressing aldehyde dehydrogenase (ALDH) activity, reflecting retinoic acid synthesis, in UC colon, both in active disease and remission, were reduced compared to controls and inflamed Crohn’s colon. In contrast, no difference in the frequency of ALDH<sup>+</sup> cells among blood precursors was detected between UC patients and non-inflamed controls. This suggests that ALDH activity in myeloid cells in the colon of UC patients, regardless of whether the disease is active or in remission, is influenced by the intestinal environment.

INTRODUCTION

Disruption of the homeostatic balance in the intestine of genetically susceptible individuals can culminate in inflammatory bowel disease (IBD), such as Crohn’s disease (CD) or ulcerative colitis (UC), where inappropriate reactivity to commensal bacteria that breach the intestinal barrier drive inflammation. Studies performed largely in mice have shown that intestinal phagocytes, such as dendritic cells (DCs) and macrophages (MQs), are central to maintaining homeostasis. In the steady state these mononuclear phagocytes are less responsive to inflammatory signals and produce anti-inflammatory mediators that promote generation of regulatory T cells (Treg).<sup>1–4</sup> However, intestinal inflammation alters the differentiation of monocyte-derived cells and changes their function into cells that promote inflammation.<sup>3–6</sup>

Compared to mice, relatively little is known about human DCs and MQs in healthy versus inflamed intestine. Intestinal MQs from healthy human intestine are hypo-responsive to inflammatory stimuli.<sup>5,7,8</sup> In the inflamed human intestine, CD14<sup>+</sup> MQs accumulate and produce proinflammatory cytokines.<sup>3,9–12</sup> Accumulation of CD14<sup>+</sup> cells is
also seen in mesenteric lymph nodes (MLNs) draining inflamed intestine.13

Similar to MQs, DCs from healthy human lamina propria are hyporesponsive to some TLR ligands14 while those from inflamed intestine exhibit proinflammatory activity.15,16 Consistent with this, CD103+ DCs from MLN draining healthy intestine promote induction of FoxP3+ T cells17 while DCs from the MLN of active CD are proinflammatory and induce Th1 T cells.18 Indeed, healthy colon epithelial cells seem to condition hyporesponsiveness in monocyte-derived DCs and promote FoxP3+ T cells, a property that is reduced in epithelial cells from non-inflamed CD patients.17,19

Studies of mouse intestinal DCs, using CD103 and CD11b to define populations, have identified distinct subsets with different origin and influence on intestinal homeostasis.4,6,20–23 The human counterparts have been identified using CD103 combined with other markers such as Sirpα or CD141 and CD1c.21,23–25 Mouse CD103+ CD11b− intestinal DCs are equivalent to CD103+ Sirpα− in the human intestine, which are highly similar to CD141+ DCs in human blood and other tissues.24 Mouse CD103− CD11b+ DC, which have a role in inducing mucosal Th17 cells,21,25,26 are related to CD103+ Sirpα+ DCs in human intestine and CD1c+ DCs in blood and skin. However, the role of these recently identified human DC subsets in IBD is not known.

Retinoic acid (RA) is a vitamin A metabolite that has many immunomodulatory properties depending on the context.27,28 It is produced from retinol in a step-wise process involving retinol dehydrogenases and aldehyde dehydrogenases (ALDH). CD103+ DCs in mouse intestinal tissue produce RA, which allows these DCs to imprint intestinal homing properties to T and B cells and promotes Treg development.1 Similar to mice, human CD103+ DC from MLN draining healthy intestine imprint intestinal homing properties to T cells in

![Image of Figure 1](https://example.com/figure1.png)

**Figure 1** DRint MQs infiltrate inflamed intestinal mucosa in CD and UC. (a) Gating strategy used to select live, Lin−HLADR−CD14− cells. (b) Lin−HLADR−CD14+ were further divided into DRhi and DRint populations. (c) The number of DRhi MQs and (e) DRint MQs among 10^5 LPMCs from the indicated tissue is shown. Control tissue for CD and UC were from ascending and sigmoid colon, respectively. Horizontal lines indicate the median. (d) The number of DRhi MQs and (f) DRint MQs among 10^5 LPMCs from non-inflamed and inflamed areas of the same patient is shown. ns = non-significant.
a RA-dependent fashion and induce FoxP3 expression in T cells. The ability of CD103+ DCs from MLN to imprint intestinal homing on T cells is similar in MLN draining healthy or inflamed ileum. Despite these findings, which subsets of mononuclear phagocytes in the human intestine have the ability to make RA and whether this is localization dependent and regulated in context of intestinal inflammation is unknown.

The pivotal role of DCs and MQs in intestinal homeostasis, and the unknown contribution of recently identified human DC subsets in IBD, led us to dissect the population dynamics of MQ and DC in inflamed and non-inflamed intestinal tissue and MLN draining these sites. Extensive patient cohorts were analyzed to compare ileum versus colon in CD and different colon segments in CD and UC. We show that MQ and DC subsets follow a specific inflammatory pattern, both in the lamina propria and MLN, which is similar at all locations and for both diseases. We also analyzed the capacity of intestinal DC and MQ to produce RA. The data suggest that intestinal tissue of UC patients influences ALDH production by myeloid cells, regardless of whether disease is active or in remission.

RESULTS

Increased HLADRint macrophages characterize inflamed intestinal mucosa

To characterize MQs infiltrating the intestinal mucosa of patients with active CD or UC, lin–HLADR+CD14+ cells were selected (Figure 1a and Supplementary Figure S1a online). HLADR expression of these cells differed in controls and inflamed patients so the cells were further divided into HLADRhi and HLADRint populations (named DRhi MQs and DRint MQs, respectively) (Figure 1b). Both DRhi MQs and DRint MQs from inflamed colon tissue showed higher expression of CD64, CD13 and Sirpα compared to control tissue, while CD209 expression was lower in DRint MQs during inflammation (Supplementary Figure 1b). No, or only moderate, differences in total DRhi MQ number (Figure 1c) or frequency (Supplementary Figure S1c) were found at inflamed sites compared to non-inflamed tissue for both ileum and colon. The same occurred when comparing inflamed to non-inflamed tissue taken from the same patient at the same endoscopy (Figure 1d). In contrast, there was a sharp increase in the number (Figure 1e) and frequency (Supplementary Figure S1d) of DRint MQs during inflammation, both compared to controls (Figure 1e and Supplementary Figure S1d) and to non-inflamed sites from the same patient (Figure 1f). Paired samples of inflamed and non-inflamed Crohn’s ileum could not be obtained due to ethical considerations. The number of DRhi MQs and DRint MQs did not differ between controls from sigmoid and ascending colon (compare CD colon control and UC colon control in Figure 1c and e). Likewise, the number of DRhi MQs and DRint MQs in controls from ileum and ascending colon were similar (compare CD ileum control and CD colon control in Figure 1c and e).

Increased DRint MQs in inflamed intestine suggests recruitment from the blood. To address this, HLADR expression on CD14+ monocytes from blood (Figure 2a) was compared to DRint and DRhi MQs among LPMCs from colon tissue (Figure 1b) from the same patient. The median fluorescent intensity (MFI) of HLADR was similar between CD14+ monocytes and DRint MQs while DRhi MQs had higher HLADR expression (Figure 2b). In summary, in Crohn’s and UC patients, recruitment of DRint MQs increased in inflammation while DRhi MQs remained constant.

CD14+ CD103+ and CD1c+ CD103+ DCs decrease in the inflamed intestinal mucosa

To further characterize antigen presenting cells in the mucosa of patients with active IBD, the CD11c+ fraction of lin–HLADR+CD14+ cells was selected and henceforth called DCs (Figure 3a, left). Using CD141 and CD1c, two separate populations could be determined, both at steady state and during inflammation (Figure 3a, middle and Supplementary Figure S2a). These cells were named CD141+ DCs and CD1c+ DCs, respectively. No double positive CD141+ CD1c+ DCs were detected in either non-inflamed or inflamed tissue (Figure 3a and data not shown). During inflammation, no change in the frequency of CD141+ DCs or CD1c+ DCs among LPMCs (Supplementary Figure S2b-c), or in the frequency among DCs, (Supplementary Figure S2d-e) was found at any location, and numbers of DCs in the ileum, ascending colon and...
sigmoid colon were similar (data not shown). CD141\(^+\) DCs and CD1c\(^+\) DCs were further analyzed for CD103 expression (Figure 3a, right). Reduced numbers of CD141\(^+\) and CD1c\(^+\) DCs expressing CD103 were detected during inflammation (Figure 3b, d). The same pattern was apparent when comparing paired samples of inflamed and non-inflamed tissue.
from the same patient at the same endoscopy (Figure 3c, e). Again, no differences in numbers of CD103<sup>+</sup> DCs between intestinal locations could be identified either during inflammation or at steady state (compare cell numbers in Figure 3b, d). Also, a reduction in the frequency of CD103<sup>+</sup> cells among the CD141<sup>-</sup>CD1c<sup>-</sup> DCs was detected upon inflammation (Supplementary Figure S3). Overall, the number and frequency of CD103<sup>+</sup> DCs among DCs was decreased during inflammation (Supplementary Figure S4). Thus, a reduction in CD103<sup>+</sup> DCs characterizes intestinal lamina propria DCs during inflammation in CD and UC.

**DC composition in MLNs shows distinct changes during inflammation**

DCs migrate to lymph nodes to initiate T cell responses. We thus compared the composition of DCs in the MLNs of patients with inflammation relative to controls. In contrast to CD14<sup>-</sup>CD11c<sup>+</sup> lamina propria DCs, where a single population was apparent (Figure 3a), MLNs contain two distinct populations of CD14<sup>-</sup>CD11c<sup>+</sup> cells, one being HLADR<sup>hi</sup> and the other HLADR<sup>int</sup> (Figure 4a). These populations were named DR<sup>hi</sup> DCs and DR<sup>int</sup> DCs, respectively. The ratio of these two populations revealed differences between MLNs from inflamed patients and controls, with DR<sup>hi</sup> DCs being the main population at steady state while DR<sup>int</sup> DCs increased during inflammation (Figure 4b).

Obtaining MLNs draining the colon of control patients is highly limited for ethical reasons. However, MLNs draining control ileum can be obtained in conjunction with bladder reconstruction. Thus, MLNs draining inflamed colon or ileum were compared to control MLNs draining the ileum. The DR<sup>hi</sup>/DR<sup>int</sup> DC ratio in MLNs draining inflamed colon from CD and UC patients was similar to MLNs draining inflamed CD ileum (Figure 4b). Also, no differences were detected regardless of whether the MLNs drained ascending, descending or sigmoid colon (Figure 4b and c; colors indicate samples from the same patient). Thus, differences in the DR<sup>hi</sup>/DR<sup>int</sup> DC ratio in MLNs draining inflamed colon compared to ileal-draining control MLNs seemed not to depend on location per se, but rather that the MLNs drained inflamed intestine. The shift from predominance of DR<sup>hi</sup> DCs in control MLNs to DR<sup>int</sup> DCs in MLNs draining inflamed IBD intestine is

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**Figure 4** Increased DR<sup>int</sup> DCs characterize MLNs draining inflamed ileum and colon. (a) Gating strategy to identify MLN DCs. Live, lin<sup>−</sup>CD45<sup>+</sup>HLADR<sup>+</sup>CD14<sup>+</sup> cells were gated for CD11c and further divided into DR<sup>hi</sup> and DR<sup>int</sup> DCs. (b) The DR<sup>hi</sup>/DR<sup>int</sup> DC ratio in the MLN draining the indicated tissue is shown. Horizontal lines indicate the median. (c) Schematic showing where the colon-draining MLNs used to generate the DR<sup>hi</sup>/DR<sup>int</sup> DC ratio in (b) were taken. Each color in (c) correlates to the DR<sup>hi</sup>/DR<sup>int</sup> DC ratio of the same color (individual) in (b). (d) DR<sup>hi</sup> DCs (blue) and DR<sup>int</sup> DCs (red) were further gated into CD141<sup>+</sup>, CD1c<sup>+</sup>, and CD141<sup>-</sup>CD1c<sup>-</sup> DCs and the median frequencies are displayed as pie charts in (e). (f) MFI of HLA DR expression on lamina propria CD141<sup>+</sup>, CD1c<sup>+</sup> and CD141<sup>-</sup>CD1c<sup>-</sup> DCs from non-inflamed and inflamed colon tissue of patients from whom MLNs were obtained (a subset of the patients shown in 4a-e; CD ileum n = 2, CD colon n = 2 and UC colon n = 2). Open triangles are ileal CD; open circles are colonic CD and closed circles are UC.
shown schematically in Figure 4d and e (compare total blue to total red).

To determine which DC subsets accounted for the shift from DRhi to DRint DCs during inflammation, MLN DCs were gated for CD141 and CD1c. About half of the numerically abundant DRhi DCs in control MLNs expressed CD1c (Figure 4e, left). These were reduced upon inflammation while DRint DCs, either expressing CD1c or being CD141−CD1c−, increased in MLNs draining inflamed tissue (Figure 4d and e). Similar trends were detected in ileal and colonic CD as well as UC. This suggests that inflammation resulted in similar relative changes in MLN DC subsets independent of the intestinal compartment or disease. Since a shift from DRhi to DRint DCs was apparent in MLNs, but control MLNs from the colon were not available to analyze for ethical reasons, we compared cell populations in the intestinal mucosa to MLNs. As stated above, no difference in the frequency of CD141+ and CD1c+ DCs in the intestinal mucosa could be detected between control and inflamed tissue (Supplementary Figure S2). We thus examined HLADR expression on mucosal DCs from patients where inflamed and non-inflamed intestinal tissues as well as MLNs were obtained. HLADR expression on CD141+, CD1c+ and CD141−CD1c− DCs was lower in inflamed compared to non-inflamed tissue of the same patient with a similar pattern for colon and ileum (Figure 4f). To further analyze HLADR expression, blood and LPMC DCs (CD141+ and CD1c+) from the same patient (UC patients in remission) were analyzed and showed that DR expression increased in the tissue (Supplementary Figure S5). Thus, the shift from DRhi to DRint DCs seen in inflamed MLNs is consistent with HLADR expression on LPMC DCs from inflamed tissue and HLADR is expressed at a higher level in tissue compare to blood.

**ALDH+ cells are reduced in colon tissue of UC patients regardless of inflammation**

The production of RA by intestinal mononuclear phagocytes has been shown to regulate Th cell differentiation and induce intestinal homing properties. As generation of RA involves ALDH enzymes, we measured ALDH activity in MQs and DCs from the intestine of CD and UC patients (Figure 5a). ALDH activity did not differ significantly between DRhi and DRint MQs of inflamed CD patients or between DRhi and DRint MQs of inflamed UC patients (compare the same patient groups in Figure 5b and e). The same was true for CD141+ and CD1c+ DCs from these patient groups as well as controls (compare the same patient groups in Figure 5d and e). However, in controls, ALDH activity was lower in DRint MQs compared to DRhi MQs (median % (range), 25% (21–49) vs. 56% (45–69), P = 0.0003; compare control colon in Figure 5b and c). In general, MQ and DC subsets from the ileum or colon of inflamed CD patients had a broad distribution in the frequency of ALDH+ cells that did not differ significantly from controls (Figure 5b-e). In contrast, ALDH activity in DCs and MQs from inflamed colon of UC patients was tightly clustered and all four subsets analyzed had a significantly reduced frequency of ALDH+ cells relative to control colon (Figure 5b-e). Similar results were found for CD103+ DCs (Supplementary Figure S6a-b). This suggests that, relative to controls, the frequency of ALDH+ myeloid cells from inflamed UC colon was reduced while ALDH+ myeloid cells from CD colon or ileum was similar to controls.

The data raised the possibility that inflamed UC colon reduces the frequency of ALDH+ cells compared to control colon. To address this, we assessed the frequency of ALDH+ cells among myeloid cells from UC patients in remission and in non-inflamed and inflamed regions of the intestine of the same UC patient with active disease. This revealed that DCs, but not MQs, showed low Aldefluor activity at remission compared to controls (Figure 5b-e, right panels). Also, a similar frequency of ALDH+ MQs and DCs were detected in non-inflamed tissue and inflamed tissue of patients with active disease (Figure 5f). Quantitative RT-PCR of sorted MQs and CD141+ and CD1c+ DCs showed similar ALDH1A1 expression in the same cell type among the different patient groups (Figure 5g). However, ALDH1A2 expression was increased in all three cell types only in inflamed CD (Figure 5g). Together the data suggest that the frequency of ALDH+ cells in the colon of UC patients with active disease is low regardless of whether the tissue is inflamed nor not. It also shows that ALDH activity is low among DCs and DRhi MQs in UC at any disease stage while a restoration of ALDH activity in DRint MQs is apparent at remission.

**Reduced ALDH+ cell frequency is inherent to the colonic mucosa of UC patients**

The ALDH data raise the possibility that the UC colon influences the frequency of ALDH-producing myeloid cells. In addition, ALDH+ CD45− (stromal) cells did not compensate for reduced RA in UC patients (Supplementary Figure S6c). We next analyzed ALDH+ cells among blood precursors (Figure 6a and b). The data revealed a high frequency of ALDH+ cells among classical and intermediate monocytes, as well as among circulating CD141+ and CD1c+ DCs, in UC patients in remission and with active disease that was similar to healthy donors (Figure 6c). ALDH activity was associated predominantly with ALDH1A1 expression that was independent of disease or disease activity (Figure 6d). Thus, the low frequency of ALDH-producing myeloid cells in the colon of UC patients in remission or with active disease is not reflected in circulating precursors. Together the data suggest that the colon environment in UC patients reduces ALDH expression of mononuclear phagocytes.

**DISCUSSION**

Using extensive clinical material encompassing Crohn’s ileitis, Crohn’s colitis and UC, as well as MLNs draining these tissues, we characterized the dynamics of MQ and DC populations, including their ability to produce RA, in health versus IBD. Our data show that CD and UC patients display the same myeloid cell imbalance in inflamed intestinal mucosa, comparisons which have not been directly made before. First, inflammation is characterized by increased CD14+ MQs in the intestinal lamina propria. These cells are CD64hi, consistent with their
This increase was accounted for by CD14+DRint cells, while CD14+DRhi cells were unchanged in inflammation. This is also consistent with studies in Crohn's patients showing an influx of CD14hi cells in inflamed intestinal mucosa that have features of less mature mononuclear cells.2,3,10–12 Second, we studied CD141+ and CD1c DCs, which have been characterized in several healthy human tissues14,21,24,31,32 but not in IBD. We found that the number of CD141+ and CD1c+ is similar in inflamed lamina propria of IBD patients and controls.

Expression of CD103, the alpha chain of the αEβ7 integrin, has been used to identify a population of DCs in mouse small intestine with a role in homeostasis.1,29,33 CD103 is not, however, a subset-specific DC marker as it is expressed to varying degrees on both IRF8/Id2/Batf3- and IRF4-dependent DCs.20–22,34 Notably, we observed a marked reduction in the frequency of CD103-expressing DCs in the setting of intestinal inflammation. This was apparent among both CD141+ and CD1c+ DCs and the so far undefined CD141−CD1c− DCs. It might be argued that although significant, the differences in the numbers of CD103-expressing cells during inflammation vs. health are small relative to the total cell count. However, the potency of DCs in controlling inflammation and tolerance is high and small numbers of DCs can have a potent influence on the ensuing response in the tissue. Inflammation-induced death of these cells, their migration from the lamina propria or negative impact of the inflammatory environment on CD103 expression may underlie reduced CD103+ DCs during inflammation. The mechanisms regulating CD103 expression on DCs subsequent to their localization in the intestine is unknown. However, CD103 expression on intestinal T cells is dependent on TGFβ.35 This raises the possibility that the inflammatory environment of IBD negatively impacts CD103 expression by DCs.

As DCs migrate from the intestinal lamina propria to MLNs to activate naïve T cells,20,34 we investigated DCs in MLNs...
draining inflamed intestine. Strikingly, we noted a marked increase in the number of DR\textsuperscript{int} DCs among CD14\textsuperscript{−}CD64\textsuperscript{−} APCs in MLNs draining inflamed intestine, regardless of which region they drained. Similar to intestinal tissue, CD1c\textsuperscript{+} DCs in MLNs draining inflamed or non-inflamed intestine were the major DC subset. However, in inflamed tissue, the CD1c\textsuperscript{+} DCs were mainly DR\textsuperscript{int} DCs and there was also a large influx of CD14\textsuperscript{−}CD1c\textsuperscript{−} DCs that were DR\textsuperscript{int}. The three DC subsets...
identified by CD103 and Sirpα2 in healthy human small intestine express CCR724 and can thus potentially migrate to MLNs. Which MLN DC subset(s) represent lamina propria-derived migratory DCs and resident DCs in the steady state remains to be determined.

RA can potentially influence intestinal homeostasis by, for example, influencing Th differentiation and inducing intestinal homing receptors on T cells.9,28,29,36 However, little is known about RALDH activity in human intestinal MQ and DC populations including how this is influenced by IBD and whether it differs in ileal CD, colonic CD and UC. We observed ALDH activity in each of the mononuclear phagocyte populations examined from control ileum and colon. This is consistent with recent studies examining DCs and MQs from healthy ileum, jejenum and colon24,37 except that we observed ALDH activity in CD141+ DCs from ileum whereas this activity was not found in CD14+ DCs from jejunum.24

Moreover, we found that the fraction of ALDH+ MQs and DCs in inflamed ileum or colon of CD was similar to controls. This contrasts with a recent study showing increased ALDH activity in CD103+ and CD103− DCs and CD14+ MQs from CD patients.37 This discrepancy could be due to how DC populations were identified in the studies. For example, Sanders et al analyzed ALDH activity in DCs gated based on the presence or absence of CD103, which does not define distinct DC populations23–25 In contrast, we analyzed DCs based on CD1c and CD141, which define distinct DC subsets.23–25 Moreover, samples obtained from different intestinal sites, different patient treatment regimens or CD complications such as strictures and fistulae could contribute to the discrepancy. Indeed, there tended to be more individual-to-individual spread in the fraction of ALDH+ cells among the CD patients whereas the UC patients behaved more homogenously. Given the diversity among CD patients with, for example, respect to disease features such as strictures or fistulae, it may be that a larger group of CD patients needs to be examined and/or their complications need to be formally considered, in addition to the use of identical gating strategies, to rectify the discrepancy. Interestingly, we found that mRNA levels of ALDH1A2 were higher in MQs and DCs from inflamed colon of CD patients compared to controls. This indicates that inflamed CD patients could have higher ALDH activity and is consistent with the data of Sanders et al.37

In contrast to inflamed CD colon, ALDH+ DCs and MQs were reduced in UC colon compared to controls. This occurred even in UC patients in remission. These data, combined with the similar fraction of ALDH+ blood monocytes in UC patients and healthy donors, suggests that ALDH activity is influenced by UC intestinal mucosa irrespective of disease activity. Consistent with Sanders et al., who showed that circulating bulk CD14+ monocytes express mainly ALDH1A1,37 we found that classical and intermediate, but not non-classical monocytes, express ALDH1A1 but little ALDH1A2. Interestingly, a large fraction of circulating CD141+ and CD1c+ DCs expressed ALDH, both in UC and controls, despite low ALDH1A1 expression. Enhanced efficiency of the oxidation of retinol to retinal by alcohol dehydrogenase family members and/or conversion of retinal to RA by RALDH enzymes, perhaps including ALDH1A3 and ALDH2, in these circulating DCs could underlie this observation.28,38 Whether circulating CD141+ and CD1c+ DCs migrate into the intestinal mucosa is unclear but transcriptional profiling studies support this possibility.24

The low ALDH activity in intestinal tissue of UC patients, despite the presence of ALDH1A1 and ALDH1A2 mRNA, could be caused by translational control, lack of factors required to maintain enzyme activity or production of negative regulators. The microbiota composition in Crohn’s disease and UC differ39,40 and the microbiota can influence metabolism in the host intestine.41 Microbiota-induced alterations in local metabolites could, in turn, differentially influence ALDH enzyme stability, translation or activity. For example, NAD+ levels are influenced by the microbiota,42 and NAD+ is a required co-factor for aldehyde dehydrogenases and is rate limiting, along with Mg2+, in the oxidation of retinol to retinoic acid.43,44 Thus, disease-associated microbiota could influence ALDH activity despite the presence of ALDH1A1 and ALDH1A2 mRNA. For negative regulation, in a study concerning cancer, niclosamide treatment reduced Wnt/β-catenin signaling.45 This resulted in increased ALDH1A1 expression and highlights the link between Wnt/β-catenin signaling and ALDH expression. Interestingly, Wnt/β-catenin signaling is induced in UC but not Crohn’s disease46 and many Wnt ligands are even upregulated in the mucosa of UC patients in remission.47 Mechanisms regulating the expression and function of enzymes involved in the oxidation of retinol in MQs and DCs in UC patients, both in the intestine and at other mucosal surfaces, warrant further investigation.

Could reduced ALDH activity in the lamina propria affect disease progression? In both human biopsies and mice, addition of RA activates the RARβ receptor and inhibits inflammatory responses and increases FoxP3+ T cells even without lymph node involvement.28,36,48 Also, all trans RA induces suppressive functions of human nTregs.49 Thus, local regulatory failure by a lack of RA may help drive the disease. However, studies in mice suggesting that RA can also enhance inflammation underscore the complex effects of RA.28 Despite the complex immunomodulatory properties of RA, it is successfully being used as therapy against psoriasis and acne and may be involved in gastric homeostasis.50–52 RA modulation is unlikely to interfere with active inflammation in UC, but may be investigated as a supplement during remission to reduce the risk of relapse.

In summary, we reveal a myeloid cell imbalance that is an inflammatory signature in the intestinal lamina propria and MLNs that is common to CD and UC. Moreover, it is similar along the colon and terminal ileum. Our data also suggest that the intestinal environment of UC influences ALDH activity of mononuclear phagocytes, irrespective of the inflammatory status of the patient per se. Down regulation of the retinoic acid system seems to be a feature of UC, active as well as inactive, and
therapeutic normalization of this phenomenon may represent a new treatment target in UC.

METHODS

Patients and specimens. UC patients, CD patients and non-inflamed controls (Table 1) were recruited during 2010-2015 at the Endoscopy unit, Sahlgrenska University Hospital, Gothenburg, Sweden, at the Department of Surgery, Östra Hospital, Gothenburg, Sweden, at the Department of Surgery, Skåne University Hospital, SUS Malmö, Sweden and at the Department of Hepatology and Gastroenterology, Aarhus University Hospital, Aarhus, Denmark. All participants gave informed written consent. The study was approved by the Regional Ethical Review Board in Gothenburg (permits 040-08 and 085-11), the Regional Ethical Board in Lund (permit 463-06) and the Local Scientific Ethical committee in Denmark (permits M-20100216 and M-20110240).

Tissue biopsies were acquired at routine colonoscopy. Inflammation was assessed macroscopically by the examining gastroenterologist and microscopically by a pathologist. Biopsies from patients without inflammation undergoing colonoscopy for other indications (polyps, weight loss) were used as non-inflamed control tissue (termed controls). Tissue and MLNs from surgery were acquired from UC and CD patients undergoing colectomy or ileectomy. Non-inflamed tissue from surgery was from patients undergoing colectomy for colon cancer and MLNs from surgery were from patients undergoing bladder reconstruction by cystectomy (both termed controls). Blood was from healthy donors, UC patients in remission and UC patients with active disease.

Blood and intestinal samples. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized venous blood by density-gradient centrifugation on Ficoll-Paque (GE Healthcare, Sweden). Biopsies and surgical samples were collected in PBS and put on ice. Surgical samples were stripped of muscle and fat and cut into small pieces. Epithelial cells were removed by incubating for 15 min at 37°C with HBSS-EDTA (HBSS containing 2% FCS, 1.5 mM Hepes and 2 mM EDTA) three times followed by a wash in RPMI 1640 containing 10% FCS and 1.5 mM Hepes. Lamina propria mononuclear cells (LPMCs) were prepared by a 45-60 min incubation at 37°C with 25 CDU/ml collagenase type VIII (Sigma-Aldrich) or 40 CDU/ml collagenase D (Roche Diagnostics) and 60 Kunitz units/ml DNase I (Sigma-Aldrich) diluted in RPMI 1640 with 10% FCS, 1.5 mM Hepes and 2 mM CaCl2. Following digestion, surgical samples were further dissociated using a GentleMACS (Miltenyi Biotec, Germany). Supernatants were collected by filtration through a nylon mesh and analyzed by flow cytometry. Cell suspensions from MLNs were obtained by cutting it into pieces and performing collagenase digestion as above for 30 min.

Flow cytometry. Cells were washed twice in HBSS containing 2% FCS, 5 mM EDTA and 20 mM HEPES and stained in the same buffer for 30 min at 4°C in the dark. 7-Aminoactinomycin D (7AAD, Sigma-
CD1c tissue to be analyzed separately. Total CD141 cells were too few from control tissue and DRhi MQs were too few from inflamed sample run in parallel.

Classical (Lin− CD14+ CD16−), intermediate (Lin− CD14+ CD16+) and non-classical (Lin− CD14lowCD16+ ) monocytes as well as CD141+ (Lin− CD14− CD16− HLA-DR+ CD141+) and CD1c+ (Lin− CD14− CD16− HLA-DR+ CD1c+) cells were sorted from peripheral blood. For Figure 5g, total CD14+ Mqs were analyzed (called DR+/DR− Mqs in Figure 5g) since DR+ Mqs were too few from control tissue and DR− Mqs were too few from inflamed tissue to be analyzed separately. Total CD14+ Mqs, CD141+ DCs and CD1c+ DCs were sorted from intestinal tissue from the indicated patient groups. Samples were sorted using a FACS Aria III (BD Biosciences). Purity was >98%.

**ALDH activity.** ALDH activity was evaluated using the Aldefluor assay (Stemcell Technologies, France) according to the manufacturer’s protocol with some modifications. Briefly, 7 × 10^6 cells were resuspended in 0.5 ml assay buffer containing 5 µl Aldefluor reagent without DEAB (15 µM). Samples were incubated at 37°C for 45 min in the dark. ALDH+ cells were identified as cells with brighter fluorescent intensity than a DEAB-inhibited sample run in parallel.

**RNA isolation and qPCR.** RNA was extracted using Nucleospin RNA kit (Macherey-Nagel GmbH, Belgium) and cDNA was produced using Quantitect Reverse Transcription Kit (Qiagen). Quantitative real-time PCR (qPCR) was performed using TaqMan Universal PCR Master Mix (Applied Biosystems). Expression of ALDH1A1 (Hs00946916_m1), ALDH1A2 (Hs00182054_m1), GAPDH (Hs03929097_g1) and HPRT (Hs01032671_m1) (all from Applied Biosystems) were quantitated using the 7500 Real Time PCR system (Applied Biosystems). Standard conditions for relative gene expression analysis as recommended by the manufacturer were applied. Expression data was normalized to GAPDH and HPRT using the 2^−ΔΔCt method.

**Statistics.** Statistical evaluations were performed using GraphPad Prism 6.0 (GraphPad Software). Wilcoxon signed rank test was used to evaluate differences between paired samples, Mann Whitney U test was performed to evaluate differences between two groups and Kruskal-Wallis test followed by Dunn’s multiple comparison was used for comparison between three or more groups.

**SUPPLEMENTARY MATERIAL** is linked to the online version of the paper at [http://www.nature.com/](http://www.nature.com/mi).
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