Macrophage and dendritic cell subsets in IBD: ALDH$^+$ 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$^+$DR^{int} MQs characterized inflamed intestinal mucosa while total CD141$^+$ or CD1c$^+$ DCs numbers were unchanged. However, CD103$^+$ DCs, including CD141$^+$CD103$^+$ and CD1c$^+$CD103$^+$ DCs, were reduced in inflamed intestine. In MLNs, two CD14$^-$ DC populations were identified: CD11c^{int}HLADR^{hi} and CD11c^{hi}HLADR^{int} cells. A marked increase of CD11c^{hi}HLADR^{int} DC, particularly DR^{int}CD1c$^+$ 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$^+$ 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).[1–4] However, intestinal inflammation alters the differentiation of monocyte-derived cells and changes their function into cells that promote inflammation.[3–6]

Compared to mice, relatively little is known about human DCs and MQs in healthy versus inflamed intestine. Intestinal MQs from healthy human intestine are hyporesponsive to inflammatory stimuli.[2,7,8] In the inflamed human intestine, CD14$^+$ MQs accumulate and produce proinflammatory cytokines.[3,9–12] Accumulation of CD14$^+$ cells is

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Received 3 November 2014; accepted 4 May 2015; published online 17 June 2015. doi:10.1038/mi.2015.48

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 ligands[14] 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 cells[17] 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.[1,27] Similar to mice, human CD103$^+$ DC from MLN draining healthy intestine imprint intestinal homing properties to T cells in

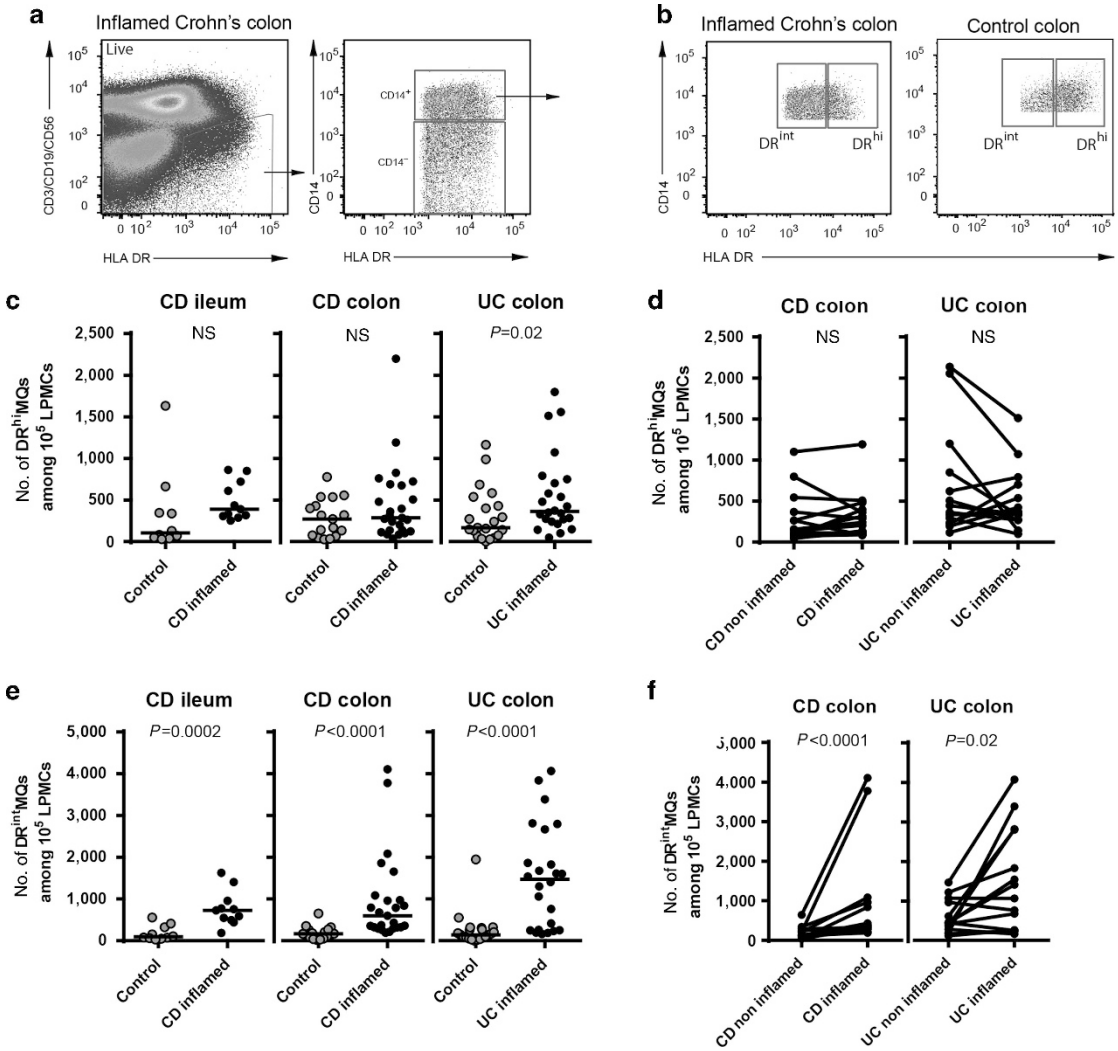

Figure 1 DR^{int} MQs infiltrate inflamed intestinal mucosa in CD and UC. (**a**) Gating strategy used to select live, Lin$^-$HLADR$^+$CD14$^+$ cells. (**b**) Lin$^-$HLADR$^+$CD14$^+$ were further divided into DR^{hi} and DR^{int} populations. (**c**) The number of DR^{hi} MQs and (**e**) DR^{int} MQs 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 DR^{hi} MQs and (**f**) DR^{int} MQs among 10^5 LPMCs from non-inflamed and inflamed areas of the same patient is shown. ns = non-significant.

a RA-dependent fashion[29] and induce FoxP3 expression in T cells.[17] The ability of CD103$^+$ DCs from MLN to imprint intestinal homing on T cells is similar in MLN draining healthy or inflamed ileum.[29] 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 HLADR^{int} 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 HLADR^{hi} and HLADR^{int} populations (named DR^{hi} MQs and DR^{int} MQs, respectively) (**Figure 1b**). Both DR^{hi} MQs and DR^{int} MQs from inflamed colon tissue showed higher expression of CD64, CD13 and Sirpα compared to control tissue, while CD209 expression was lower in DR^{int} MQs during inflammation (**Supplementary Figure 1b**). No, or only moderate, differences in total DR^{hi} 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 DR^{int} 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 DR^{hi} MQs and DR^{int} 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 DR^{hi} MQs and DR^{int} MQs in controls from ileum and ascending colon were similar (compare CD ileum control and CD colon control in **Figure 1c** and **e**).

Increased DR^{int} MQs in inflamed intestine suggests recruitment from the blood. To address this, HLADR expression on CD14$^+$ monocytes from blood (**Figure 2a**) was compared to DR^{int} and DR^{hi} 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 DR^{int} MQs while DR^{hi} MQs had higher HLADR expression (**Figure 2b**). In summary, in Crohn's and UC patients, recruitment of DR^{int} MQs increased in inflammation while DR^{hi} MQs remained constant.

CD141$^+$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

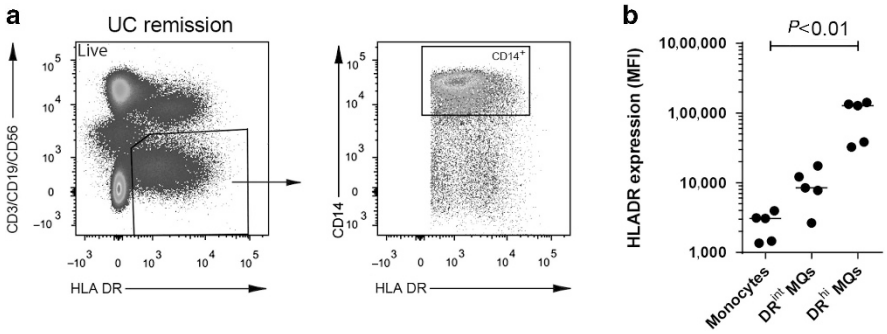

Figure 2 HLADR expression on intestinal DR^{int} MQs resembles CD14$^+$ blood monocytes. (**a**) Gating strategy used to select live, Lin$^-$CD14$^+$ blood cells (monocytes). (**b**) Median fluorescence intensity (MFI) of HLADR on CD14$^+$ monocytes and intestinal DR^{int} and DR^{hi} MQs. Paired blood and tissue samples were obtained from five UC patients in remission. Horizontal lines indicate the median.

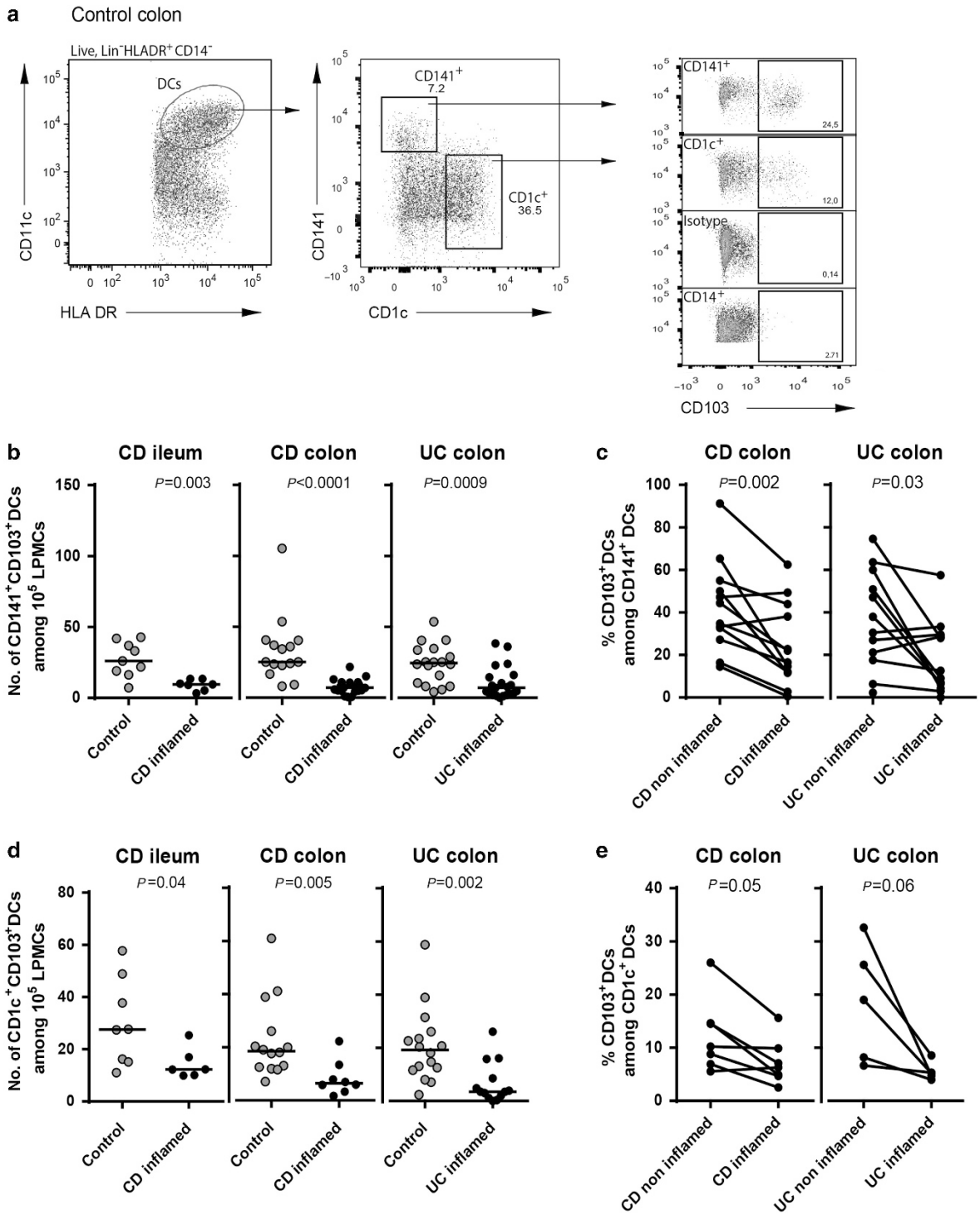

Figure 3 Lamina propria CD141$^+$CD103$^+$ and CD1c$^+$CD103$^+$ DCs decrease during inflammation. (**a**) Gating strategy for DCs. The CD14$^-$ population in **Figure 1a** was further gated as CD11c$^+$ (left). These cells were then divided into CD141$^+$ and CD1c$^+$ DC populations (middle) and further gated into CD141$^+$CD103$^+$ and CD1c$^+$CD103$^+$ DCs (right). Isotype control for CD103 (total DC population) and CD103 control staining of DR^{hi} MQs are shown. The number of CD141$^+$ CD103$^+$DCs (**b**) and CD1c$^+$CD103$^+$DCs (**d**) among 10^5 live LPMCs from the indicated tissue is shown. Control samples are as in **Figure 1**. Horizontal lines indicate the median. The frequency of CD103$^+$ cells among CD141$^+$ DCs (**c**) and CD1c$^+$ DCs (**e**) from non-inflamed and inflamed areas of the same patient is shown.

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$^+$ 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$^+$ cells among the CD141$^-$CD1c$^-$ DCs was detected upon inflammation (**Supplementary Figure S3**). Overall, the number and frequency of CD103$^+$ DCs among DCs was decreased during inflammation (**Supplementary Figure S4**). Thus, a reduction in CD103$^+$ 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 MLN of patients with inflammation relative to controls. In contrast to CD14$^-$CD11c$^+$ lamina propria DCs, where a single population was apparent (**Figure 3a**), MLNs contain two distinct populations of CD14$^-$CD11c$^+$ cells, one being HLADR^{hi} and the other HLADR^{int} (**Figure 4a**). These populations were named DR^{hi} DCs and DR^{int} DCs, respectively. The ratio of these two populations revealed differences between MLNs from inflamed patients and controls, with DR^{hi} DCs being the main population at steady state while DR^{int} 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^{hi}/DR^{int} DC ratio in MLNs draining inflamed colon from CD and UC patients was very 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^{hi}/DR^{int} 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^{hi} DCs in control MLNs to DR^{int} DCs in MLNs draining inflamed IBD intestine is

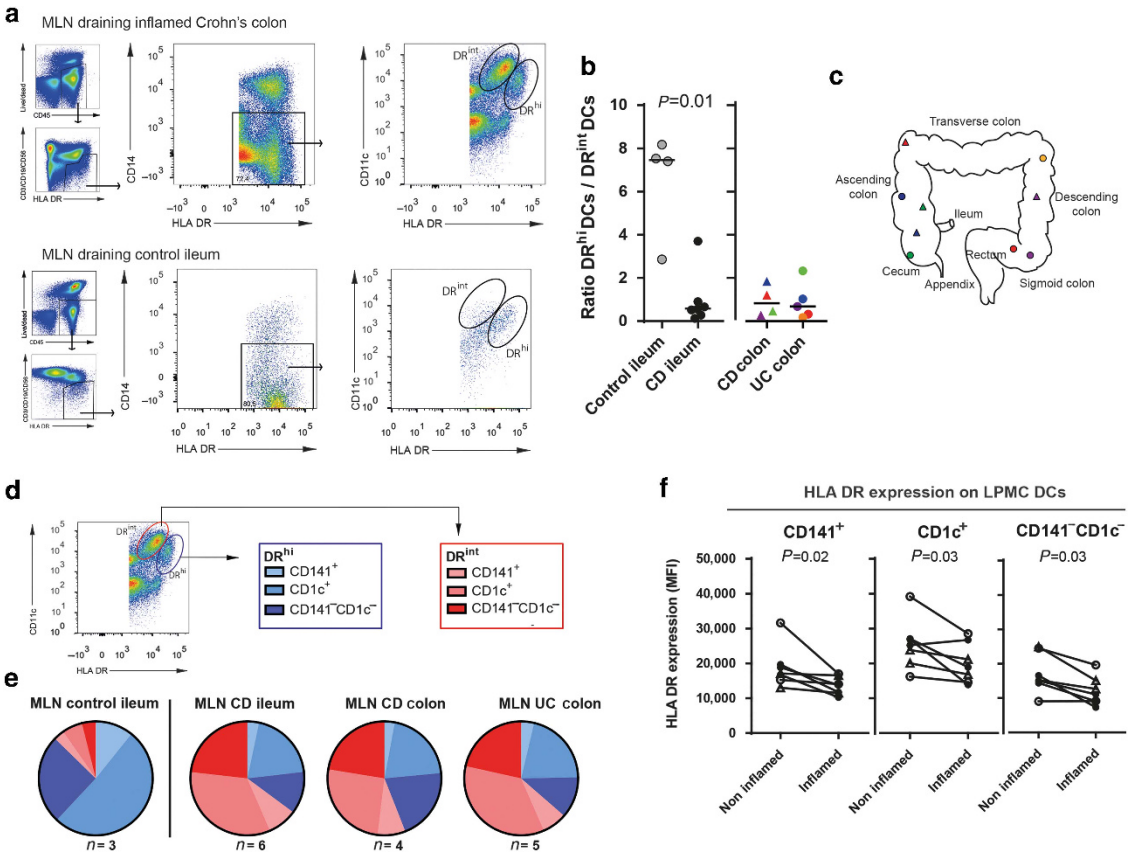

Figure 4 Increased DR^{int} DCs characterize MLNs draining inflamed ileum and colon. (**a**) Gating strategy to identify MLN DCs. Live, lin$^-$CD45$^+$HLADR$^+$CD14$^-$ cells were gated for CD11c and further divided into DR^{hi} and DR^{int} DCs. (**b**) The DR^{hi}/ DR^{int} DC ratio in the MLN draining the indicated tissue is shown. Horizontal lines indicate the median. (**c**) Schematic showing where the colon-draining MLNs used to generate the DR^{hi}/DR^{int} DC ratio in (**b**) were taken. Each color in (**c**) correlates to the DR^{hi}/ DR^{int} DC ratio of the same color (individual) in (**b**). (**d**) DR^{hi} DCs (blue) and DR^{int} DCs (red) were further gated into CD141$^+$, CD1c$^+$ and CD141$^-$CD1c$^-$ DCs and the median frequencies are displayed as pie charts in (**e**). (**f**) MFI of HLADR expression on lamina propria CD141$^+$, CD1c$^+$ and CD141$^-$CD1c$^-$ DCs from non-inflamed and inflamed colon tissue of patients from whom MLNs were also 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 DR^{hi} to DR^{int} DCs during inflammation, MLN DCs were gated for CD141 and CD1c. About half of the numerically abundant DR^{hi} DCs in control MLNs expressed CD1c (**Figure 4e**, left). These were reduced upon inflammation while DR^{int} 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 DR^{hi} to DR^{int} 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 DR^{hi} to DR^{int} 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 suggested to regulate Th cell differentiation and induce intestinal homing properties.[27,28] 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 DR^{hi} and DR^{int} MQs of inflamed CD patients or between DR^{hi} and DR^{int} MQs of inflamed UC patients (compare the same patient groups in **Figure 5b** and **c**). 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 DR^{int} MQs compared to DR^{hi} 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 DR^{hi} MQs in UC at any disease stage while a restoration of ALDH activity in DR^{int} 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 $CD64^{hi}$, consistent with their

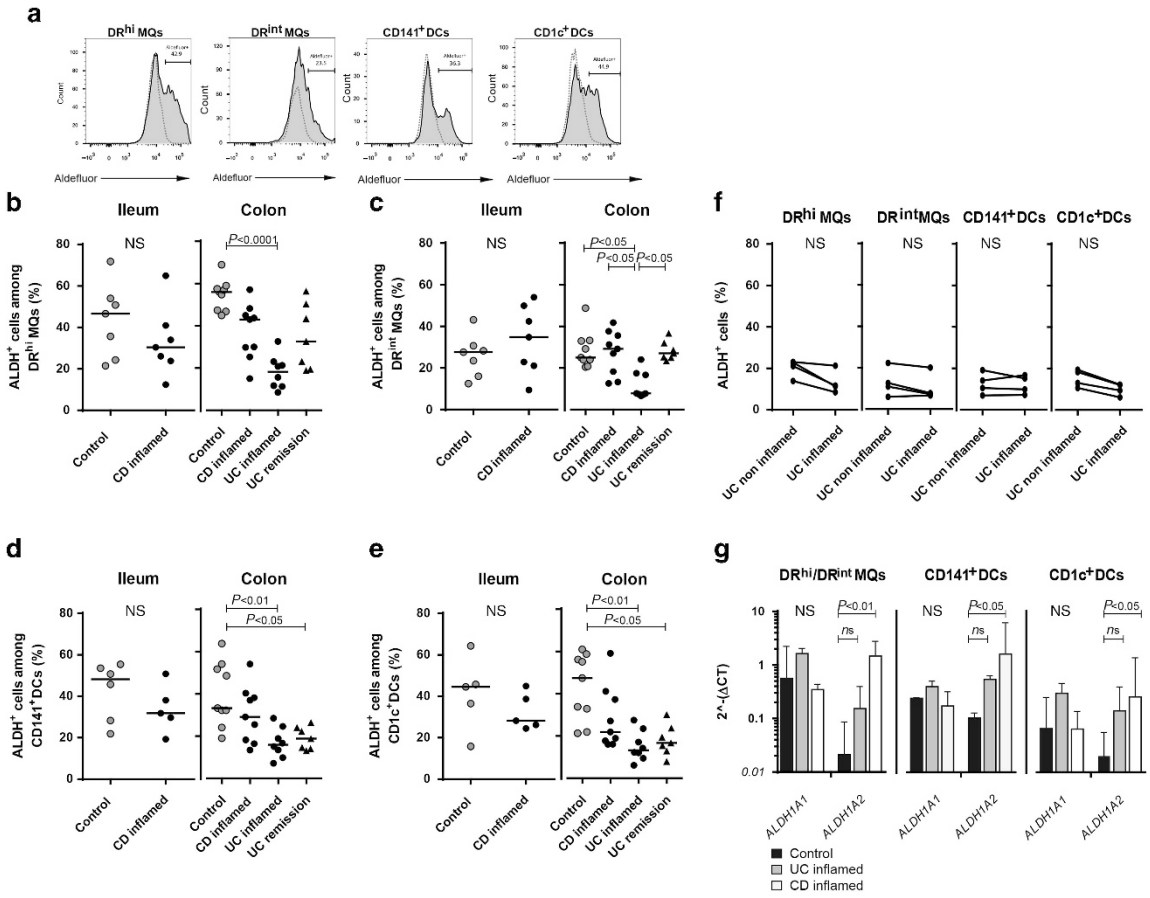

Figure 5 Low frequency of ALDH$^+$ DCs and MQs characterizes inflamed UC lamina propria. (**a**) Histograms of ALDH$^+$ cells among the indicated populations from the colon of an inflamed CD colitis patient is shown. Cells were incubated with ALDEFLUOR in the absence (filled) or presence (dotted line) of DEAB inhibitor. The percent Aldeflour$^+$ cells is shown above the horizontal line indicating the positive gate. The frequency of ALDH$^+$ cells among DR^{hi} MQs (**b**), DR^{int} MQs (**c**), CD141$^+$ DCs (**d**) and CD1c$^+$ DCs (**e**) in inflamed tissue (CD and UC) and in remission (UC) compared to controls is shown. Horizontal lines indicate the median. (**f**) The frequency of ALDH$^+$ cells among DR^{hi} MQs, DR^{int} MQs, CD141$^+$ DCs and CD1c$^+$ DCs from non-inflamed and inflamed areas of the same patient is shown. (**g**) RT-PCR analysis of *ALDH1A1* and *ALDH1A2* expression relative to *HPRT* by total CD14$^+$ MQs (called DR^{hi}/DR^{int} MQs in the figure), CD141$^+$ DCs and CD1c$^+$ DCs purified from intestinal tissue by sorting using the gates in **Figures 1a** and **3a** is shown (control $n=4$, UC $n=4$ and CD $n=4$). Bars show the median and range. Statistical significance is indicated with the *P*-value and all other comparisons were non-significant.

identity as MQs.[3,6,30] This increase was accounted for by CD14$^+$DR^{int} cells, while CD14$^+$DR^{hi} cells were unchanged in inflammation. This is also consistent with studies in Crohn's patients showing an influx of CD14^{hi} 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 tissues[14,21,24,31,32] 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

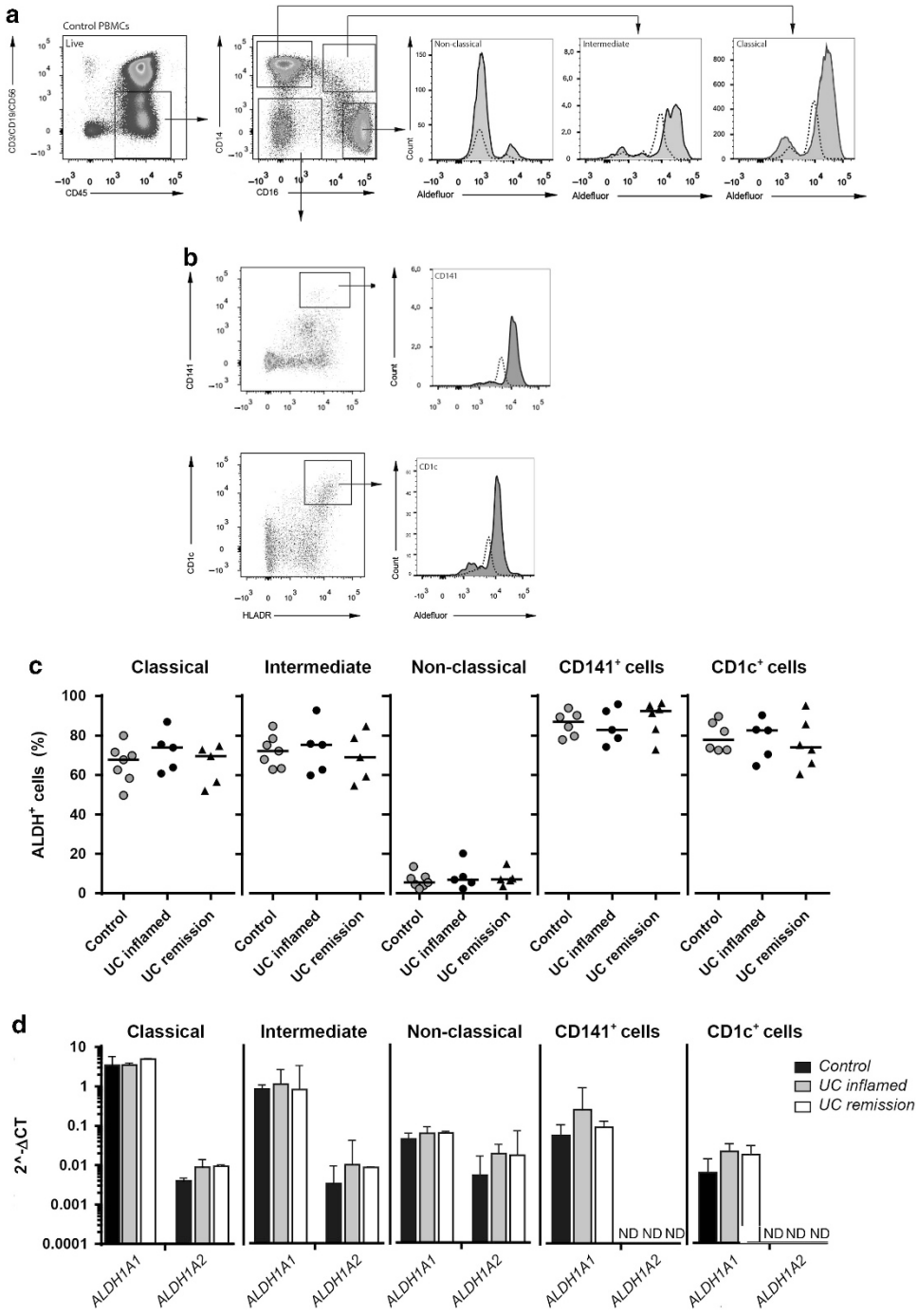

Figure 6 Similar frequency of circulating ALDH$^+$ cells in UC patients and healthy donors. (**a**,**b**) Gating strategy showing PBMCs of a healthy donor. Live, Lin$^-$HLADR$^+$CD45$^+$ cells were further gated into (**a**) non-classical (CD14^{low}CD16$^+$), intermediate (CD14$^+$CD16$^+$) and classical (CD14$^+$CD16$^-$) monocytes and CD14$^-$CD16$^-$ cells. (**b**) The double negative cells were further gated into CD141$^+$ and CD1c$^+$ cells. ALDH$^+$ cells were identified as in **Figure 5a**. The frequency of ALDH$^+$ cells among (**c**) classical, intermediate and non-classical monocytes as well as CD141$^+$ and CD1c$^+$ cells from the blood of healthy donors, UC patients with active disease and UC patients in remission is shown. Horizontal lines indicate the median. (**d**) RT-PCR analysis of *ALDH1A1* and *ALDH1A2* expression relative to *HPRT* by the indicated cell populations purified from the blood of healthy donors ($n=5$), UC patients with active disease ($n=4$) and UC patients in remission ($n=3$) by sorting using the gates in (**a**,**b**). nd = not detected. Bars show median and range. All comparisons were non-significant.

draining inflamed intestine. Strikingly, we noted a marked increase in the number of DR^{int} DCs among CD14$^-$CD64$^-$ APCs in MLNs draining inflamed intestine, regardless of which region they drained. Similar to intestinal tissue, CD1c$^+$ DCs in MLNs draining inflamed or non-inflamed intestine were the major DC subset. However, in inflamed tissue, the CD1c$^+$ DCs were mainly DR^{int} DCs and there was also a large influx of CD141$^-$CD1c$^-$ DCs that were DR^{int}. The three DC subsets

identified by CD103 and Sirpα in healthy human small intestine express CCR7[24] 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, jejunum and colon[24,37] except that we observed ALDH activity in CD141$^+$ DCs from ileum whereas this activity was not found in CD141$^+$ 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 populations[23–25] 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 differ,[39,40] 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 Mg^{2+}, 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 disease[46] 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 RARα 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

Table 1 Patient demographics

	UC	*CD*	*Controls*
Total number of patients	45	51	39
Male/female	34/11	27/24	21/18
Age	43 (20–76)[a]	38 (22–80)	60 (18–90)
Smoking habit (active/ex smoker/never)	4/6/35	7/7/35[b]	8/8/23
Disease duration, years	9 (1–48)	12 (1–55)	NA[c]
Disease status (active inflammation/remission)	36/9	51/0	NA
Examination (endoscopy/surgery/blood only)	27/9/9	34/17/0	21/10/8
Samples obtained (colon/ileum/MLN/blood)	36/0/5/15	31/20/11/0	25/12/4/8
Treatments:			
Corticosteroids, thiopurines, anti-TNF	2	3	0
Corticosteroids, 5-ASA, anti-TNF	3	0	0
Corticosteroids, 5-ASA, thiopurines	2	0	0
Corticosteroids, thiopurines	0	1	0
Corticosteroids, 5-ASA	4	1	0
Corticosteroids, anti-TNF	0	1	0
Thiopurines, anti-TNF	0	1	0
Thiopurines, 5-ASA	2	4	0
Anti-TNF	0	2	0
Corticosteroids	1	4	1
Thiopurines	1	15	0
5-ASA	22	7	0
Neoadjuvant chemotherapy	0	0	2
No treatment	8	12	36

[a]Data are shown as median (range).
[b]Data from 2 patients are missing.
[c]NA, not applicable.

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 $CaCl_2$. 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 but 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-

Aldrich) or Live/Dead Fixable Aqua Dead Cell Stain Kit (Gibco Life Technologies) were used to exclude non-viable cells. Antibodies used were: anti-HLADR-Alexa700/APC-Cy7, anti-CD3-PE-CF594/FITC, anti-CD19-PE-CF594/FITC, anti-CD56-PE-CF594, anti-CD11c-Pacific Blue/PeCy7, anti-CD14-PeCy7/FITC, anti-CD103-PE/APC, anti-CD141-PE, anti-CD1c-PerCP-Cy5.5, anti-CD45-APCH7, anti-CD16-APC, anti-CD64-APC, anti-CD13-APC, anti-CD33-APC, anti-CD209-APC and anti-Sirpα-PeCy7. Antibodies were from BD Biosciences (San Jose, CA) except anti-CD103 (eBioscience), anti-HLA-DR (BioLegend), anti-CD1c (BioLegend) and anti-CD141 (Miltenyi). Samples were collected with a LSRII- or FACSCanto (BD Biosciences) using DIVA software (BD Biosciences) and analyzed using FlowJo software (Tree Star). Lineage cocktail was anti-(CD3/CD19/CD56). Isotype controls were used for anti-CD14-PeCy7/FITC, anti-CD103-PE/APC, anti-CD141-PE, anti-CD1c-PerCP-Cy5.5, anti-CD64-APC, anti-CD13-APC, anti-CD33-APC, anti-CD209-APC and anti-Sirpα-PeCy7.

Classical ($Lin^-CD14^+CD16^-$), intermediate ($Lin^-CD14^+CD16^+$) and non-classical ($Lin^-CD14^{low}CD16^+$) monocytes as well as $CD141^+$ ($Lin^-CD14^-CD16^-HLADR^+CD141^+$) and $CD1c^+$ ($Lin^-CD14^-CD16^-HLADR^+CD1c^+$) cells were sorted from peripheral blood. For **Figure 5g**, total $CD14^+$ MQs were analyzed (called DR^{hi}/DR^{int} MQs in **Figure 5g**) since DR^{int} MQs were too few from control tissue and DR^{hi} 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 or with 10 µl of the ALDH inhibitor 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* (Hs00180254_m1), *GAPDH* (Hs03929097_g1) and *HPRT* (Hs01003267_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 $2^{-\Delta Ct}$ 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/mi

ACKNOWLEDGMENTS

We thank Drs Jan Marsal and Olof Grip and Nurses Lisbeth Eklund, Ann-Louise Helminen and Hillevi Björkqvist for their support in providing clinical samples. This work was supported by LUA-ALF Clinical Research Grants, The Swedish Cancer Foundation, AFA Insurance, National Institute of Allergy and Infectious Disease (U01AI095473, subaward 9006862_Got_C1; the content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH), The Danish Crohn's and Colitis Foundation, ABBvie Pharmaceuticals, Arla Foods, Denmark and the foundations of Assar Gabrielsson, Ruth and Richard Julin, Åke Wiberg, Lars Hierta, Wilhelm and Martina Lundgren, Längmanska Kultur, and the Swedish Society of Medicine.

DISCLOSURE

J.A. is an advisory board member of AbbVie Pharmaceuticals, Ferring Pharmaceuticals and a speaker for ABBvie, MSD. Biopsies from patients at Århus University Hospital were obtained in an investigator-initiated study supported in part by funding from AbbVie, Denmark and Arla Foods, Denmark. All other authors disclose no competing interest. Study sponsors had no role in the study design, sample collection, data analysis or data interpretation.

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