MyD88 and Retinoic Acid Signaling Pathways Interact to Modulate Gastrointestinal Activities of Dendritic Cells

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BACKGROUND & AIMS: Gut-associated dendritic cells (DC) metabolize vitamin A into *all-trans* retinoic acid (RA), which is required to induce lymphocytes to localize to the gastrointestinal tract and promotes the differentiation of Foxp3$^+$ regulatory T cells and IgA antibody–secreting cells. We investigated whether RA functions in a positive-feedback loop in DC to induce its own synthesis. **METHODS:** We measured levels of retinoids in intestinal tissues from mice and assessed the role of RA in the functional specialization of gut-associated DC in cell cultures and mice. We used pharmacologic antagonists to determine the signaling pathways involved in regulation of DC and used *MyD88*$^{-/-}$ mice to determine the contribution of Toll-like receptor signaling in RA-mediated effects on DC. **RESULTS:** The concentration of retinoids decreased in a proximal-to-distal gradient along the intestine, which correlated with the activity of gut-specific DC. Importantly, RA regulated the ability of gut-associated DC to produce RA, induce T cells to localize to the gastrointestinal tract, and generate regulatory T cells and IgA-secreting cells. RA was sufficient to induce its own production by extraintestinal DC in vitro and in vivo. RA-mediated regulation of DC required signaling through the mitogen-activated protein kinase signaling pathway and unexpectedly required MyD88, which is conventionally associated with Toll-like receptor, interleukin-1, and interleukin-18 signaling. **CONCLUSIONS: RA is necessary and sufficient to induce DC to regulate T-cell localization to the gastrointestinal tract and IgA secretion. Our findings also indicate crosstalk between the RA receptor and MyD88-dependent Toll-like receptor signaling pathways.**

Keywords: Inflammation; IBD; Immune Response; Mouse Model.

Lymphocyte migration is a key event during intestinal inflammation.[1] Therefore, it is critical to understand how T and B cells migrate to the intestine and how their migration patterns could be manipulated for therapeutic purposes. The gut-homing receptors integrin $\alpha4\beta7$ and chemokine receptor CCR9 are required for T- and B-lymphocyte migration to the gut mucosa in the steady state and also during intestinal inflammation in mice and humans,[1,2] thus acting as molecular "ZIP codes" by controlling lymphocyte migration in a tissue-specific fashion.

DC from mesenteric lymph nodes (MLN-DC), Peyer's patches (PP-DC), and small intestine lamina propria (gut-associated DC), but not DC from extraintestinal sites, induce a high expression of gut-homing receptors on lymphocytes,[3–6] which is explained by their ability to metabolize vitamin A (retinol) into all-*trans* retinoic acid (RA). RA is necessary to imprint gut-homing lymphocytes,[5–7] to promote differentiation of IgA antibody-secreting cells (ASC)[5] and to control the balance between regulatory T cells and Th_{17} cells in the gut mucosa.[8,9] Therefore, given the essential role of RA in intestinal immune homeostasis, it is important to understand how the synthesis of RA is modulated in the gut mucosa.

It has been suggested that some nuclear receptor agonists can modulate RA synthesis in DC.[10] Here we show that RA controls RA-synthesizing capacity in DC in vitro and in vivo, inducing a positive feedback loop on its own synthesis and conferring DC with gut-specific imprinting properties. In addition, we found that RA-mediated DC education requires expression of the intracellular adaptor MyD88, which is conventionally associated with Toll-like receptor (TLR) and interleukin (IL)-1/IL-18 signaling,[11,12] suggesting a novel crosstalk between RA- and MyD88-dependent pathways.

Abbreviations used in this paper: ASC, antibody-secreting cells; DC, dendritic cells; ERK, extracellular signal–regulated kinase; GM-CSF, granulocyte-macrophage colony-stimulating factor; IEC, intestinal epithelial cells; IL, interleukin; LP-DC, lamina propria–derived dendritic cells; MAPK, mitogen-activated protein kinase; MLN-DC, mesenteric lymph node–derived dendritic cells; Mo-DC, monocyte-derived dendritic cells; mRNA, messenger RNA; PLN-DC, peripheral lymph node–derived dendritic cells; PPAR, peroxisome proliferator-activated receptor; PP-DC, Peyer patches–derived dendritic cells; RA, *all-trans* retinoic acid; RA-DC, dendritic cells pretreated with all-*trans* retinoic acid; RALDH, retinal dehydrogenase; RAR, retinoic acid receptor; RXR, retinoid X receptor; TLR, Toll-like receptor; UT-DC, untreated spleen dendritic cells; VAD, vitamin A–deficient.

0016-5085/$36.00
doi:10.1053/j.gastro.2011.04.010

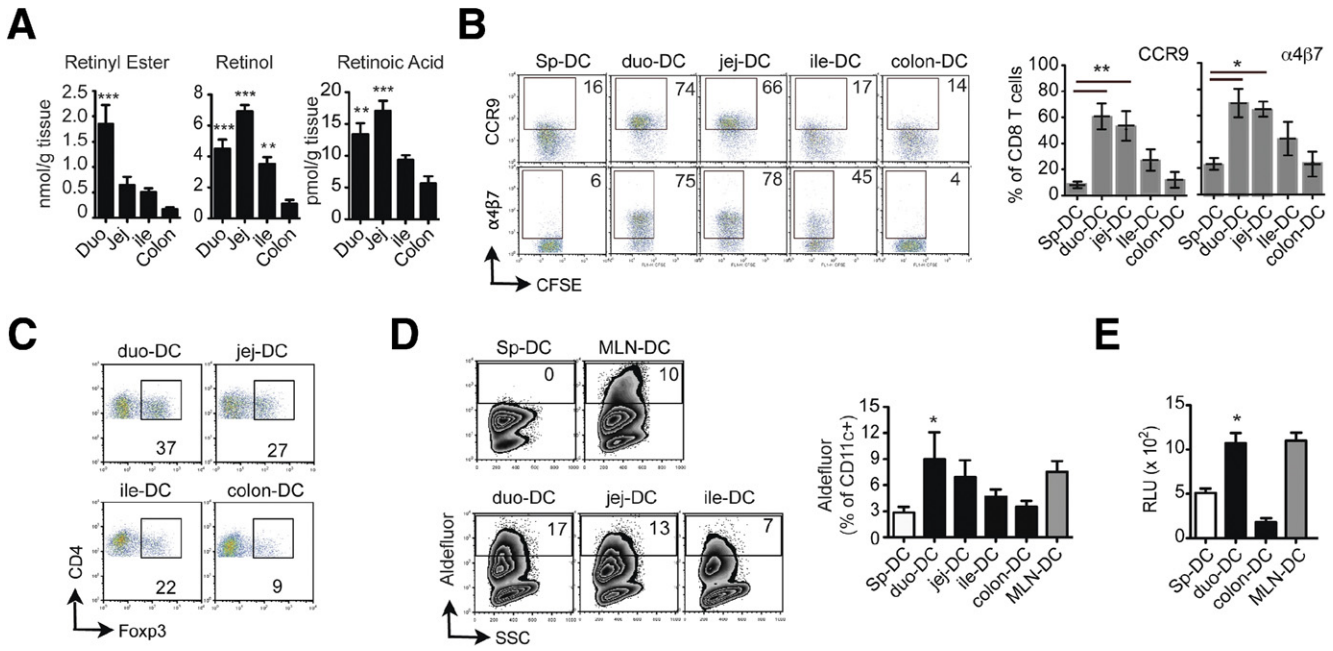

Figure 1. DC ability to imprint gut homing correlates with retinoid levels in the gut. (*A*) Quantification of retinyl esters, retinol, and RA in duodenum (duo), jejunum (jej), ileum (ile), and colon (n = 6). (*B*) LP-DC or PP-DC from duo, jej, ile, and colon were used to activate naïve CD8 T cells. After 4−5 days, T cells were analyzed for $\alpha 4\beta 7$ and CCR9 expression (n = 5). (*C*) DC were used to activate naïve CD4 T cells. After 4 days, T cells were analyzed for Foxp3 expression. Fluorescence-activated cell sorting (FACS) plots are representative of 2 independent experiments. (*D*) Retinal dehydrogenase (RALDH) activity in DC (n = 4). (*E*) Luciferase activity in DC from DR5-luciferase mice (n = 4). Mean ± SEM; $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$.

Results

DC Ability to Imprint Gut Homing Correlates With Retinoid Levels in the Gut

RA and its precursors retinol and retinyl esters were readily detected in the intestinal mucosa, and their concentrations followed a gradient from proximal to distal, with the highest concentrations being found in the duodenum and jejunum (Figure 1*A*). Interestingly, retinoid levels correlated with DC imprinting ability. PP-DC and lamina propria DC (LP-DC) from duodenum and jejunum induced higher levels of gut-homing receptors ($\alpha 4\beta 7$ and CCR9) and Foxp3^{+} T cells as compared to their counterparts in ileum, colon, or spleen (Figure 1*B* and *C*, Supplementary Figure 1A). Reciprocally, the skin-homing receptors E- and P-selectin ligands were more efficiently induced by ileum PP-DC/LP-DC, colon LP-DC, and spleen-DC than by duodenum or jejunum PP-DC/LP-DC (Supplementary Figure 1*B*), consistent with the notion that induction of these receptors occurs as a default pathway in T cells activated without RA.[7] Retinal dehydrogenases (RALDH) are critical enzymes for RA biosynthesis. Consistent with their higher gut-homing imprinting capacity, PP-DC and LP-DC from duodenum and jejunum exhibited higher RALDH activity (Aldefluor staining) than ileum or colon DC (Figure 1*D*), which was not explained by different proportions of CD103^{+} DC (Supplementary Figure 1*C*). Of note, expression of *Aldh1a2* messenger RNA (mRNA) (encoding RALDH2) was not significantly different among DC from different intestinal segments and their relative mRNA levels were lower than those found in MLN-DC (Supplementary Figure 1*D*), suggesting that *Aldh1a2* mRNA levels in DC do not always fully correlate with their RALDH activity or gut-homing imprinting capacity. Whether this dissociation between *Aldh1a2* mRNA and RALDH activity reflects local differences in RALDH protein expression or stability remains to be determined. In addition, using DR5-luciferase reporter mice, in which luciferase is controlled by a promoter with RA response elements,[13] we determined that all tested DC have the capacity to respond to RA ex vivo (Supplementary Figure 1*E*). However, consistent with their exposure to high levels of RA, DC from the proximal small intestine exhibited higher luciferase activity than distal DC (Figure 1*E*).

RA Is Necessary in vivo for Gut-Associated DC Education

We depleted mice of the RA precursor retinol by feeding them a vitamin A–deficient (VAD) diet, as described.[5,7] Because vitamin A is abundantly stored in the liver, it is difficult to attain complete vitamin A depletion, even when using a VAD diet for several months.[14] To address this shortcoming, we used mice deficient in lecithin:retinol acyltransferase (LRAT), which cannot store retinol in the liver.[14] LRAT-deficient mice develop normally when maintained on a vitamin A−sufficient diet, but they become vitamin A−depleted after only 2−4 weeks on a VAD diet, with the additional advantage of avoiding potential unwanted effects of chronic vitamin A depletion.[14] In agreement with a critical role of RA in gut-associated DC education, PP-DC and MLN-DC from VAD mice induced lower levels of gut-homing receptors