European Journal of Immunology

WILEY-VCH

European Journal of Immunology
Basic • Clinical • Translational

RESEARCH ARTICLE OPEN ACCESS

Immunodeficiencies and Autoimmunity

Type 1 and CD103$^+$ Type 2 Conventional Dendritic Cells Are Decreased in Active Patients with Ulcerative Colitis but Not with Crohn's Disease

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Received: 15 January 2025 | **Revised:** 1 December 2025 | **Accepted:** 3 December 2025

Keywords: conventional dendritic cells | human | inflammatory bowel disease

ABSTRACT

This study aimed to characterize human intestinal conventional dendritic cells (cDCs) in health and inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD). Three cDC subsets (CD103$^-$ cDC2, CD103$^+$ cDC2, and cDC1) were identified from lamina propria mononuclear cells. Their phenotype and function were analyzed in healthy and IBD-inflamed gut tissues. In the healthy gut, cDC2 predominated over cDC1, with CD103$^+$ cDC2 dominating the duodenum and CD103$^-$ cDC2 prevalent in the ileum and colon. CD103$^+$ cDC2 expressed higher PD-L1 and produced more IL-10. In culture, CD103$^+$ cDC2 increased proportionally unless inhibited by LPS. All subsets induced IL-10$^+$ helper T-cell differentiation, with ileal cDCs being more stimulatory than colonic ones. In IBD, cDCs showed constitutively lower SIRPα expression across conditions. Notably, UC-inflamed colon exhibited reduced cDC1 and CD103$^+$ cDC2, while CD-inflamed colon maintained these subsets but showed increased T-cell stimulation and IL-17$^+$ T-cell priming. Intestinal cDC subsets prime IL-10$^+$ helper T-cells in health. In UC, reduced cDC1 and CD103$^+$ cDC2 in inflamed mucosa contrast with CD, suggesting distinct pathogenic mechanisms that could inform targeted therapies.

Abbreviations: APC, antigen-presenting cells; CD, Crohn's disease; cDC, conventional dendritic cells; DC, dendritic cells; GI, gastrointestinal; IBD, inflammatory bowel disease; pDC, plasmacytoid dendritic cells; UC, ulcerative colitis.

José A. Garrote and Javier P. Gisbert contributed equally to this work as senior authors.

KEY SUMMARY

Established knowledge on the subject

- Conventional dendritic cells (cDCs) in the intestinal lamina propria play a critical role in maintaining immune homeostasis and modulating immune responses.
- cDC subsets, including CD103$^+$ and CD103$^-$ cDC2 and cDC1, are differentially distributed across intestinal segments.
- Dysregulation of cDC subsets has been implicated in inflammatory bowel disease (IBD), both Crohn's disease (CD) and ulcerative colitis (UC).
- The ability of cDCs to influence T-cell responses, including IL-10$^+$ helper T-cell priming, is key to regulating inflammation and maintaining gut health.

Significant and/or new findings of the study

- **Regional distribution and function**: CD103$^+$ cDC2 dominate the duodenum, exhibiting anti-inflammatory properties (elevated PD-L1 and IL-10 production), while CD103$^-$ cDC2 are predominant in the colon and ileum.
- **Impact of IBD**: UC and CD demonstrate distinct alterations in cDC subsets, with UC specifically showing a reduction in cDC1 and CD103$^+$ cDC2 in inflamed colonic tissue, unlike CD.
- **T-cell priming differences**: Colonic cDCs in CD-inflamed tissue are more stimulatory, priming IL-17$^+$ T-cells, highlighting a proinflammatory tendency.
- **Unique pathogenic mechanisms**: These findings suggest differing immune dysregulation mechanisms between UC and CD, contributing to the unique inflammatory profiles of each disease.

1 | Introduction

Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), is a chronic disorder of unknown aetiology that involves a pathological response of the immune system resulting in chronic inflammation of the gastrointestinal (GI) tract. IBD affects 7 million people globally, and its prevalence is continuously increasing, especially in industrialized regions, including Europe and North America [1]. Although IBD incidence varies widely depending on the different countries, it is increasing rapidly, probably due to the "westernization" of lifestyles [2].

Dendritic cells (DC), the most potent antigen-presenting cells (APC), determine the outcome (proinflammatory or tolerogenic) of antigen-specific adaptive immune responses [3, 4]. DC are divided into two major subsets, named conventional or classical DC (cCD) and plasmacytoid DC (pDC). In the GI-tract, cDC are essential to maintain the balance between tolerance toward nutrients/commensals and immunity against pathogens [5–7]. cDC can be further divided into subsets based on their ontogeny and function, named type 1 (cDC1), specialized in cross-presentation, and type 2 (cDC2), specialized in classical presentation [8–10]. cDC1 require transcription factors IRF4 and Notch2. In mice, they express surface marker CD8α, although in humans, they are characterized by the expression of CD141. Nevertheless, both mice and human cDC1 express XCR1 and CD103. cDC2, on the contrary, requires transcription factor IRF8 and Baft3. While murine cDC2 express surface marker CD11b, in humans they are characterized by the expression of CD1c, although both species express SIRPα. The gut, however, harbors a unique subset of CD103$^+$ cDC2. This gut-specific population controls most of the mechanisms of immune tolerance, given its unique capacity to generate gut-homing regulatory T cells (Treg) and IgA-producing B cells, although they can also drive Th17 responses [11–13].

While human GI-cDC show a tolerogenic function which helps to maintain immune homeostasis, in IBD patients, they display a proinflammatory biased profile [14–24]. Indeed, GI-cDC from IBD patients have a reduced capacity to generate suppressor Treg cells coupled with an enhanced production of proinflammatory Th1 and Th17 effector T-cells, which is thought to be associated with lower numbers of CD103$^+$ cDC [15, 24, 25]. Nevertheless, although cDC are likely to be essential in IBD development and progression, it is currently unknown whether these differences in cDC biology in IBD are restricted to any specific subset. Moreover, the properties of the immune system [26, 27], including cDC subset composition [28–30], systematically change through its length. However, despite UC exclusively affecting the human colon while CD can happen anywhere in the GI-tract (from mouth to anus), few studies have attempted to study GI-cDC in both CD and UC, abrogating those regional differences. To that end, we here specifically studied human intestinal cDC from patients with CD or UC, either active or quiescent, and compared them with the noninflamed colon from the same patients and with controls.

2 | Material and Methods

2.1 | Patients and Biological Samples

Blood (10 mL) and intestinal biopsies from healthy controls were obtained during colonoscopy from a total of 56 healthy controls (35.7% males; 53.8 ± 11.8 years [mean ± standard deviation]; age interval 33–80). Patients had been referred due to rectal bleeding, dyspepsia, or colorectal cancer screening. Paired samples were obtained from the distal colon, proximal colon, and the terminal ileum (when accessible) from the same patients. Duodenal samples were also obtained in the context of an upper endoscopy from five noninflamed controls (60% males, 42.2 ± 8.2 years; age interval 25–66) referred to endoscopy for *Helicobacter pylori* screening, all of them negative for the infection. All controls had macroscopically and histologically normal mucosa with no known inflammatory, autoimmune, or malignant disease at the time of the endoscopy or colonoscopy. A maximum of eight biopsies were obtained per tissue/patient. Samples were immediately preserved in ice-chilled complete medium (Dutch modified RPMI 1640 [Sigma-Aldrich, Dorset, UK] containing 100 µg/mL penicillin/streptomycin, 2 mM L-glutamine, 50 µg/mL gentamicine [Sigma-Aldrich], and 10% foetal calf serum [TCS Cellworks, Buckingham, UK]) and processed within 30 min.

Blood and colonic biopsies were also obtained from IBD patients, including 15 patients with active UC (defined by a Mayo endo-

scopic score >1; Table S1), 11 patients with quiescent UC (defined by a Mayo endoscopic score ≤1; Table S2), 12 patients with active CD (defined by a simplified endoscopic activity score for CD [SES-CD] score > 3; Table S3) and 7 patients with quiescent CD (defined by a SES-CD score ≤3; Table S4). In the case of patients with active disease (either UC or CD), both the inflamed and the noninflamed colonic mucosa were sampled.

In addition, ileal and colonic resections were obtained from the proximal and distal ends of patients with colorectal cancer, with a minimum distance of 10 cm to the tumors (Table S5). In a similar manner, the affected and nonaffected tissue was also obtained from patients with CD subjected to tissue resection (Table S6).

Last, but not least, blood samples from healthy controls were also obtained from "Biobanco del Centro de Hemoterapia y Hemodonación de Castilla y León" (Valladolid, Spain).

In all cases, samples were obtained following written informed consent after ethical approval from the Ethics Committee at La Princesa Hospital (Madrid, Spain) and Hospital Clínico Universitario (Valladolid, Spain).

2.2 | Blood Processing

Peripheral blood mononuclear cells (PBMC) were obtained by centrifugation over Ficoll-Paque PLUS (Amersham Biosciences, Chalfont St. Giles, UK). PBMC were washed twice in PBS containing 1 mM ethylenediaminetetraacetic acid (EDTA) and 0.02% sodium azide (FACS buffer) and stained with fluorochrome-conjugated antibodies as explained below.

2.3 | Biopsy Processing

Intestinal biopsies were processed as previously described [31]. Briefly, intestinal biopsies were processed to obtain lamina propria mononuclear cells (LPMC) following two incubations (30 min each) with Hanks balanced salt solution (HBSS) (Gibco BRL, Paisley, Scotland, UK) containing 1 mM DTT and 1 mM EDTA solutions to remove the associated mucus/bacteria and epithelial layer, respectively, and further digested in the presence of 1 mg/mL of collagenase D and 20 μg/mL of Liberase (Roche Diagnostics Ltd, Lewes, UK). LPMC were subsequently passed through a 100 μm cell strainer and collected by centrifugation before they were further used for flow cytometry staining or culture (5 million LPMC in 2.5 mL of complete medium during 18 h) in the presence/absence of LPS (100 ng/mL, Sigma-Aldrich).

2.4 | Tissue Resections Processing

Human intestinal resections were collected immediately following surgery in ice-chilled RPMI medium (4°C). Tissue was cleaned with HBSS, and muscle and fat were removed using surgical scissors. As long as the tissue was clean, it was cut into pieces of about 1 cm^2 to process them separately in 15 mL tubes. First, tissue was incubated with 5 mL Hank's buffered salt solution HBSS supplemented with 1 mM DTT and 1 mM EDTA in an orbital shaker (30 min, 250 rpm, 37°C). Following incubation, the supernatant was discarded, and the remaining tissue was incubated under the same conditions to remove the mucus layer, enterocytes, and intraepithelial leukocytes. Remaining tissue was subsequently digested in 5 mL of RPMI medium supplemented with collagenase D (1 mg/mL), Liberase (20 μg/mL), and Benzonase (25 U/mL) in an orbital shaker (three incubations, 30 min each, 250 rpm, 37°C). Following each incubation, the medium must be filtered with a 100 μm strainer to obtain LPMC, which were further preserved at 4°C until used. The remaining tissue was further digested two more times following the same approach. Following incubations, all LPMC were collected in the same tube, which was further centrifuged (300*g*, 10 min, 4°C) and resuspended in RPMI before they were cryopreserved in freezing media (FBS supplemented with 10% DMSO) until used.

2.5 | Human Colonic Cytokine Milieu

Cell-free culture supernatant from the biopsy cultures was collected and stored at −80°C until analysed. Before analysis, samples were centrifuged to remove any debris. Levels of IFN-γ, IL-10, IL-17A, IL-1β, IL-6, TNF-α, and IL-8 were determined using MILLIPLEX MAP custom magnetic bead panel kit following the manufacturer's specifications. A broad sensitivity range of standards was used to help enable the quantitation of a wide dynamic range of cytokine concentrations while still providing high sensitivity. Median fluorescent intensity was calculated using the MAGPIX system version (Luminex), which integrates the Luminex xPOTENT acquisition software and the MILLIPLEX Analyst 5.1 analysis software. Values above or under the standard curve for each cytokine (IFN-γ: 0.61–2500 pg/mL; IL-10: 0.88–6000 pg/mL; IL-17A: 0.73–3000 pg/mL; IL-1β: 0.49–2000 pg/mL; IL-6: 0.8–2500 pg/mL; IL-8: 0.31–2000 pg/mL; TNF-α: 0.43–1750 pg/mL) were reported as equal to them, respectively.

2.6 | Antibody Labeling

Cells were stained with monoclonal antibodies and characterized by flow cytometry. In all cases, a live/dead fixable near-IR dead cell stain kit (Molecular Probes) was added to the cells before performing antibody staining, hence allowing the exclusion of dead cells from the analysis. Table S7 shows the specificity, clone, fluorochrome, and source of the antibodies used. Cells were labeled in FACS buffer on ice and in the dark for 20 min following Fc block incubation (Becton Dickinson). For the assessment of intracellular cytokines, cells were permeabilized (Leucoperm, Abd Secrotec) following surface staining and stained with intracellular antibodies. cDC endocytic activity was determined by uptake of TRITC-dextran (molecular weight 40 kDa, 100 μg/mL, 30 min at 37°C or on ice; Sigma) followed by subsequent surface staining of the cells as above. In all cases, cells were further washed in FACS buffer, fixed with 2% paraformaldehyde in FACS buffer for 10 min on ice, and washed again in FACS buffer before they were stored at 4°C before acquisition on the flow cytometer. For cell sorting, LPMC were immediately acquired following staining in FACS buffer and collected in complete medium.

2.7 | T-Cell Enrichment and Stimulation

Naïve T-cells were enriched from total PBMCs from buffy coats (provided by the *Biobanco del Centro de Hemoterapia y Hemodonación de Castilla y León*) using magnetic beads following the manufacturer's instructions (Pan T Cell Isolation Kit human, Miltenyi Biotec) and further stained with CellTrace Violet following the manufacturer's instructions in AIM-V medium. Different subsets of intestinal sorted cDC subsets were used to stimulate naïve T cells in a 1/20 proportion in round-bottomed 96-well plaques for 5 days in AIM-V medium. In all cases, experiments included a negative (naïve T cells cultured in resting conditions) and a positive control (T cells stimulated in the presence of 1 μg/mL phytohemagglutinin). Naïve T cells were also cultured with paired intestinal Mϕ from the same donor as a second negative control. Following the culture, T cells were further stained as previously explained.

2.8 | Flow Cytometry and Data Analysis

LPMC and PBMC were acquired on an LSR-Fortessa (BD Biosciences) or on a FACS Aria for cell sorting. Following T-cell stimulation, cells were acquired on a Gallios (Beckman Coulter). Results were analyzed using FlowJow (version 10.1) within singlet viable cells. Positive and negative gatings were set by the fluorescence minus one (FMO) method. Of note, and as opposed to other human intestinal APC, like monocytes and macrophages [32], human intestinal cDC subsets do not display differences in their FMO.

2.9 | Statistical Analysis

T-test or one/two-way ANOVA (with or without repeated measures) and subsequent Tukey or Sidak ad hoc correction were applied using GraphPad (Prism v8). The specific test applied for each analysis is detailed in each figure legend. The level of significance was fixed at $p < 0.05$ in all cases.

3 | Results

3.1 | CD103$^+$ cDC2 were the Main Subset in the Human Duodenum but Not in the Colon or the Ileum

Human intestinal cDC were identified within singlet viable leukocytes as CD14$^-$CD64$^-$HLA-DR$^+$CD11c$^+$ (Figure 1A). Given that the properties of the immune system vary throughout its length [26, 27], we first determined cDC densities in different compartments of the human gut, revealing that their proportion was higher in the large bowel (proximal and distal colon) compared with the small bowel (terminal ileum and duodenum; Figure 1B).

cDC were divided into subsets based on the expression of CD103 and SIRPα. Hence, cDC1 were identified as CD103$^+$SIRPα$^-$ while cDC2 were identified as SIRPα$^+$. The latter were further divided into CD103$^+$ and CD103$^-$ cDC2 (Figure 1C). Further analysis confirmed that cDC1 were CD141$^+$CD1c$^-$XCR1$^+$ while cDC2 were CD141$^-$CD1c$^+$XCR1$^-$ (not shown) in agreement with previous observations [30, 32, 33]. The proportion of cDC1 and cDC2 did not change throughout the human gut (Figure 1D). Further analysis within the cDC2 subset revealed that CD103$^+$ cDC2 were predominant in the duodenum as opposed to the CD103$^-$ cDC2 fraction, which was predominant in the lower compartments of the GI-tract, including the colon and the terminal ileum (Figure 1E).

3.2 | cDC1 and CD103$^+$ cDC2 Were More Mature Than Their CD103$^-$ cDC2 Counterparts

We next characterized cDC subsets in the human colon. cDC1 and CD103$^+$ cDC2 were typically more mature than their CD103$^-$ cDC2 counterparts as they had higher levels of CD40, CD137L and ICOSL. HLA-DR and CD86, on the contrary, were preferentially expressed by CD103$^+$ cDC2. Finally, although CCR7 expression displayed much variability, it was preferentially expressed by cDC1 (Figure 2A).

We also assessed PD-L1 expression on human intestinal cDC as it mediates the generation of Treg cells [34, 35]. Although PD-L1 expression is scarce on human intestinal cDC, its expression was restricted to CD103$^+$ cDC2 (Figure 2B). Fc receptor CD16 was also associated with CD103$^+$ cDC2 (Figure 2B). However, although M-DC8/SLAN is associated with CD16$^+$ APC [36], M-DC8/SLAN was not detected on human intestinal cDC or macrophages (MΦ) (data not shown). Scavenger receptors were also differentially expressed between subsets. Hence, although both CD163 and CD206 were found on cDC2, CD163 was preferentially expressed on the CD103$^-$ cDC2 fraction as opposed to CD206, which was mainly found on the CD103$^+$ cDC2 subset (Figure 2B). Finally, although all cDC subsets were endocytic, that was overall higher on CD103$^+$ cDC2 (Figure 2C).

3.3 | The Proportion of CD103$^+$ cDC2 Was Increased Following Overnight Culture

The ontogeny of CD103$^+$ cDC2 remains unknown, although they are likely derived from newly arrived CD103$^-$ cDC2 following mucosal conditioning [33]. In this regard, the proportion of cDC1 and cDC2 DC was not altered following LPMC overnight culture, either in resting conditions or in the presence of LPS (Figure 3A,B). However, within cDC2, the proportion of CD103$^+$ cells was increased after culture, although this was prevented in the presence of proinflammatory LPS (Figure 3A,B).

3.4 | IL-10 Production Was Related to CD103$^+$ DC2

We next assessed the cytokine profile of the different mucosal cDC subsets. While IL-1β production was associated with cDC2, both in resting conditions as well as in the presence of LPS, IL-23 was not produced by any particular cDC subset (Figure 4). TNFα production, on the contrary, was mainly produced by CD103$^+$ cDC2. Finally, IL-10 was produced by all human intestinal cDC subsets, although its production was higher on CD103$^+$ cDC2.

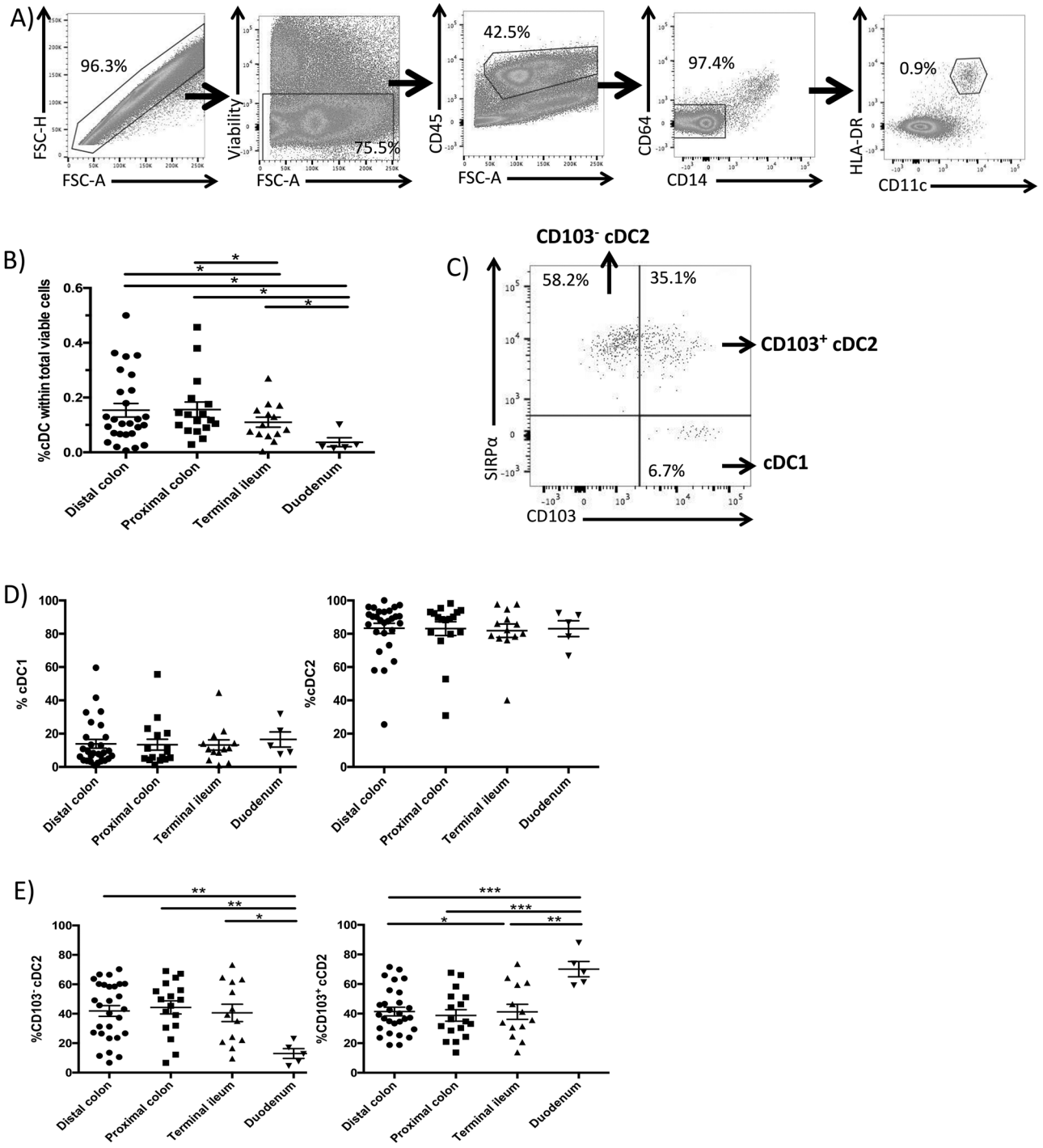

FIGURE 1 | Human intestinal conventional dendritic cell subsets. (A) Human intestinal conventional dendritic cells (cDC) were identified within singlet viable leukocytes ($CD45^+$) as $CD14^-CD64^-HLA\text{-}DR^+CD11c^+$ on lamina propria mononuclear cells (LPMC) by flow cytometry. (B) The proportion of cDC (referred to the total number of viable LPMC) was lower in the duodenum (compared with the ileum, proximal colon, and distal colon) and the terminal ileum (compared with both the proximal and distal colon). (C) Total cDC were divided into subsets based on the expression of CD103 and SIRPα. Hence, type 1 cDC (cDC1) were identified as $CD103^+SIRP\alpha^-$ while type 2 (cDC2) were identified as $SIRP\alpha^+$. cDC2 were also divided into subsets based on the expression of CD103. (D) cDC1 and cDC2 did not change their proportions through the human gut. (E) However, within the cDC2 subset, $CD103^+$ cDC2 were the main subset in the duodenum as opposed to the ileum or the colon, where $CD103^-$ DC2 were the majority. For (B, D, E) samples from the distal colon, proximal colon, and terminal ileum were obtained from the same controls (when access to the ileum was available) in the course of a colonoscopy, while duodenal samples were obtained from independent donors since they were obtained in the course of an upper endoscopy. Results from (B, D, E) also denote samples from the same individuals, considered either total cDC (B) or divided into subsets (D, E). One-way ANOVA repeated measures and subsequent Tukey´s correction (B, D, E) was applied to compare cDC between the distal colon, proximal colon, and terminal ileum, while duodenal samples were compared with the other three by *t*-test. *p*-values <0.05 were considered significant (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

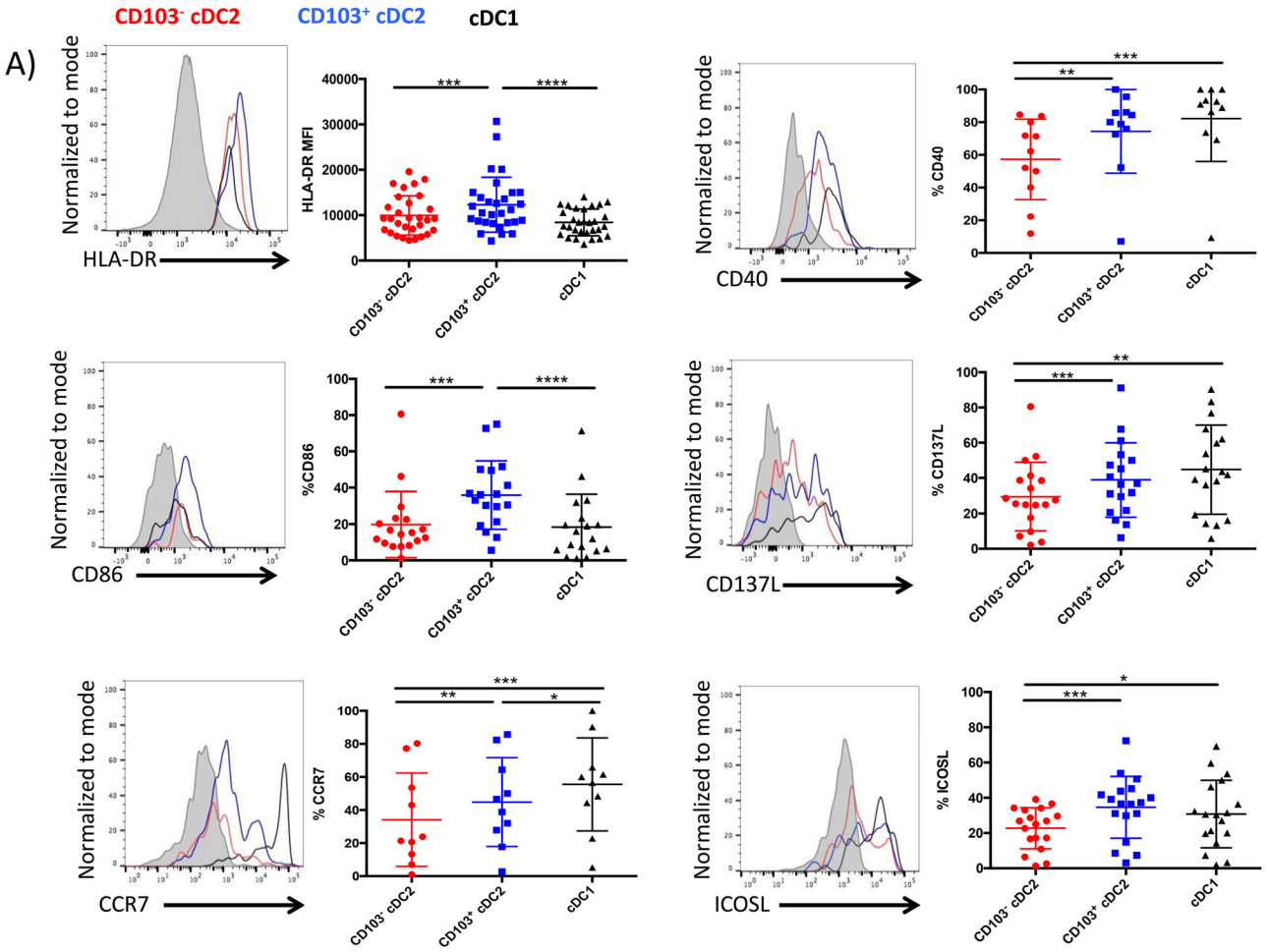

FIGURE 2 | Characterization of conventional dendritic cell subsets. (A) Human colonic conventional dendritic cell (cDC) subsets from controls were identified as in Figure 1C and characterized for the expression of HLA-DR, CD40, CD86, CD137L, ICOSL, and CCR7; as well as (B) PD-L1, CD163, CD206, and CD16. (C) DC subset phagocytic capacity was also assessed. Histograms show representative levels of expression of each marker in each given subset. Proportion of positive cells for each given marker within each cDC subset (shown on the pooled plots) was determined by the region method referred to specific fluorescence minus one (FMO) controls (shaded histograms, after confirming no FMO differences for the three different cDC subsets) for all the markers excluding HLA-DR (were shaded histogram denotes its expression on the CD11c^{-}HLA-DR^{-} fraction within lamina propria viable leukocytes) and Dextran (were shaded histogram shows the phagocytic capacity of cell preserved as 4°C). One-way ANOVA repeated measures with Tukey´s correction was applied in all cases. *p*-values <0.05 were considered significant (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$).

3.5 | All Human Intestinal cDC Subsets Prime the Generation of IL-10^{+} T-Cells in the Noninflamed Colon

Having addressed human intestinal cDC phenotype in resting conditions, we next studied their stimulatory capacity in the human setting. To that end, human intestinal CD103^{-} cDC2, CD103^{+} cDC2, and cDC1 subsets were sorted in parallel to total Mϕ as previously described [31], and their stimulatory capacity, together with the acquired profile of the stimulated T-cells, was determined (Figure S1A). Our results confirmed that all human intestinal cDC subsets can stimulate human allogenic naïve T-cells, as opposed to intestinal macrophages (Figure 5A). Besides, all human intestinal cDC subsets (including cDC1) primed mainly the proliferation of CD4^{+} T-cells (Figure 5B). Indeed, the 3 intestinal cDC subsets primed the generation of IL-10^{+} T-cells, being this capacity expanded in the cDC1 and CD103^{+} cDC2 with little or no production of IFNγ or IL-17A (Figure 5C). In addition, given that the properties of human intestinal cDC change along the length of the GI-tract [28–30], we also determined whether that also translates into a different function between ileal and colonic cDC. Hence, Figure 5D proves that all three ileal cDC subsets are more stimulatory in the ileum than in the colon, although the type (Figure 5E) or cytokine profile (Figure 5F) of the responding T-cells is not affected by cDC origin.

3.6 | SIRPα Expression Was Decreased on Mucosal cDC from IBD Patients, while the Proportion of cDC1 and CD103^{+} cDC2 Was Lower in the Inflamed Colon from UC but Not CD Patients

Having characterized the phenotype and function of human intestinal cDC in health, they were further studied in IBD. Given that the proportion of cDC (Figure 1B), together with their subset composition (Figure 1E) and stimulatory capacity (Figure 5E), are

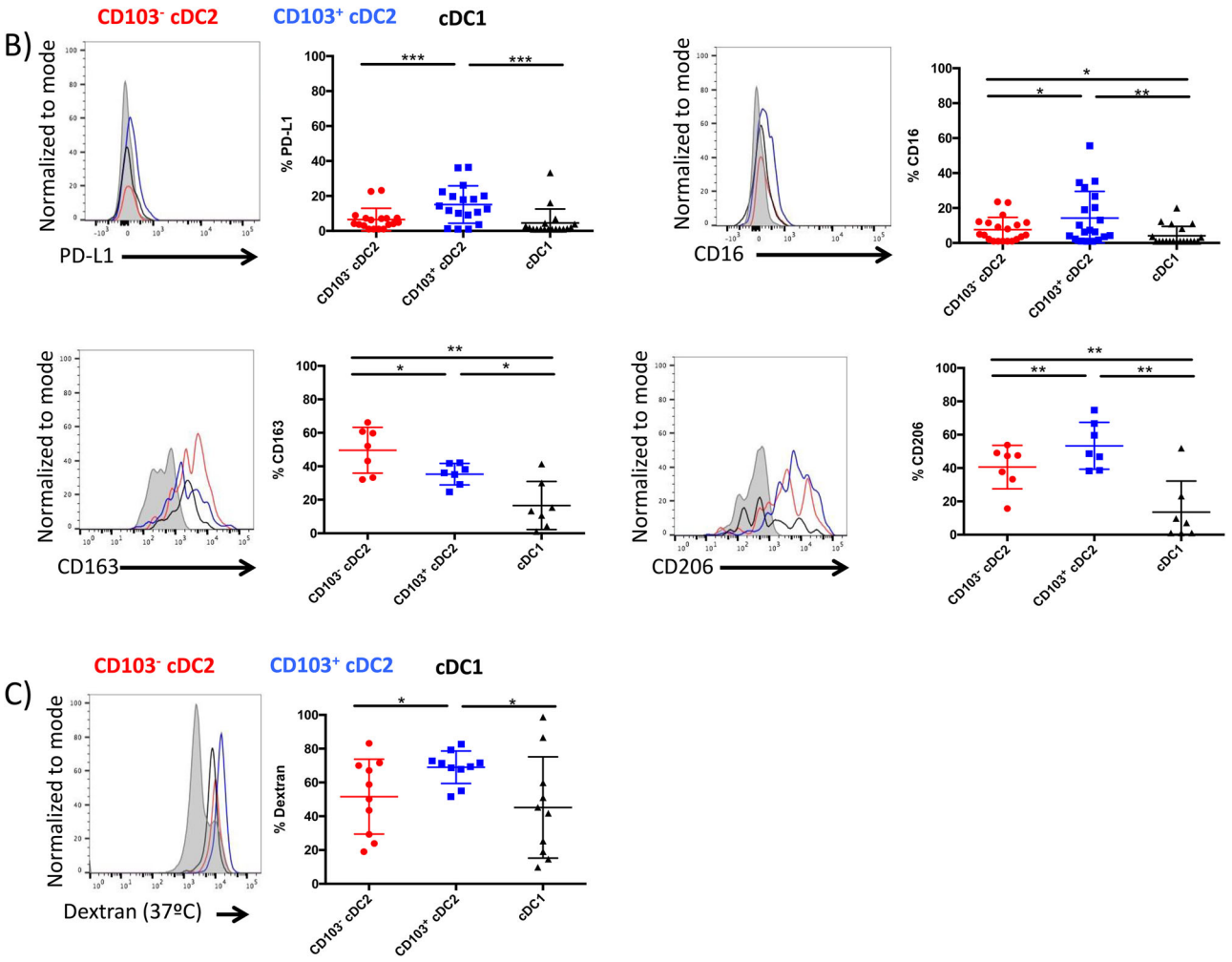

FIGURE 2 | (Continued)

influenced by the tissue under study, we here specifically focused on the human colon, hence abrogating regional differences.

The proportion of total colonic cDC (Figure 6A) was not altered in the IBD mucosa. Nevertheless, colonic cDC from IBD patients constitutively displayed lower expression of SIRPα irrespective of the IBD type (UC/CD) or status (inflamed/noninflamed; Figure 6B,C). Further analysis showed that the proportion of cDC1 and CD103$^+$ cDC2 was specifically lower in the inflamed colon from patients with active UC but not CD (Figure 6D). Consequently, the inflamed tissue from these patients also carried higher numbers of CD103$^-$ cDC2. Moreover, the inflamed mucosa from patients with active UC (and to a lower extent the noninflamed tissue from the same patients) also displayed higher numbers of CD103$^-$SIRPα$^-$ putative cDC (Figure 6D).

3.7 | Dysregulated Phenotype on Intestinal cDC Subsets from IBD Patients

We next assessed cDC phenotype in the IBD mucosa. HLA-DR expression was decreased on CD103$^+$ cDC2 in the inflamed colon from patients with CD and UC compared with the healthy mucosa (Figure 7). Activation markers CD40 and ICOSL were increased on cDC2 from patients with active disease (CD or UC), although CD40 was higher in the CD103$^-$ fraction as opposed to ICOSL, which was expanded in the CD103$^+$ subset (Figure 7). CD137L was associated with active CD as it was higher in all three subsets (CD103$^-$ cDC2, CD103$^+$ cDC2, and cDC1) as well as on CD103$^+$ cDC2 from patients with quiescent CD (Figure 7). On the contrary, CD86 was associated with inflamed UC as it was increased on all cDC subsets from these patients, as well as on CD103$^-$ cDC2 in CD. Finally, inhibitor receptor PD-L1 was ubiquitously upregulated on all intestinal cDC subsets in the inflamed colon from IBD patients.

3.8 | cDC Stimulatory Capacity in IBD

Having shown that human intestinal cDC subsets display an altered composition and phenotype in IBD patients (which was, however, more prominent in UC patients), we next determined whether that also translated into a different function. Given, however, that UC patients barely have resections, the stimulatory capacity of human intestinal cDC subsets was therefore restricted to CD patients.

Our results suggested that, in agreement with the altered phenotype observed in the noninflamed tissue from CD patients, this also translated into an increase in stimulatory capacity as

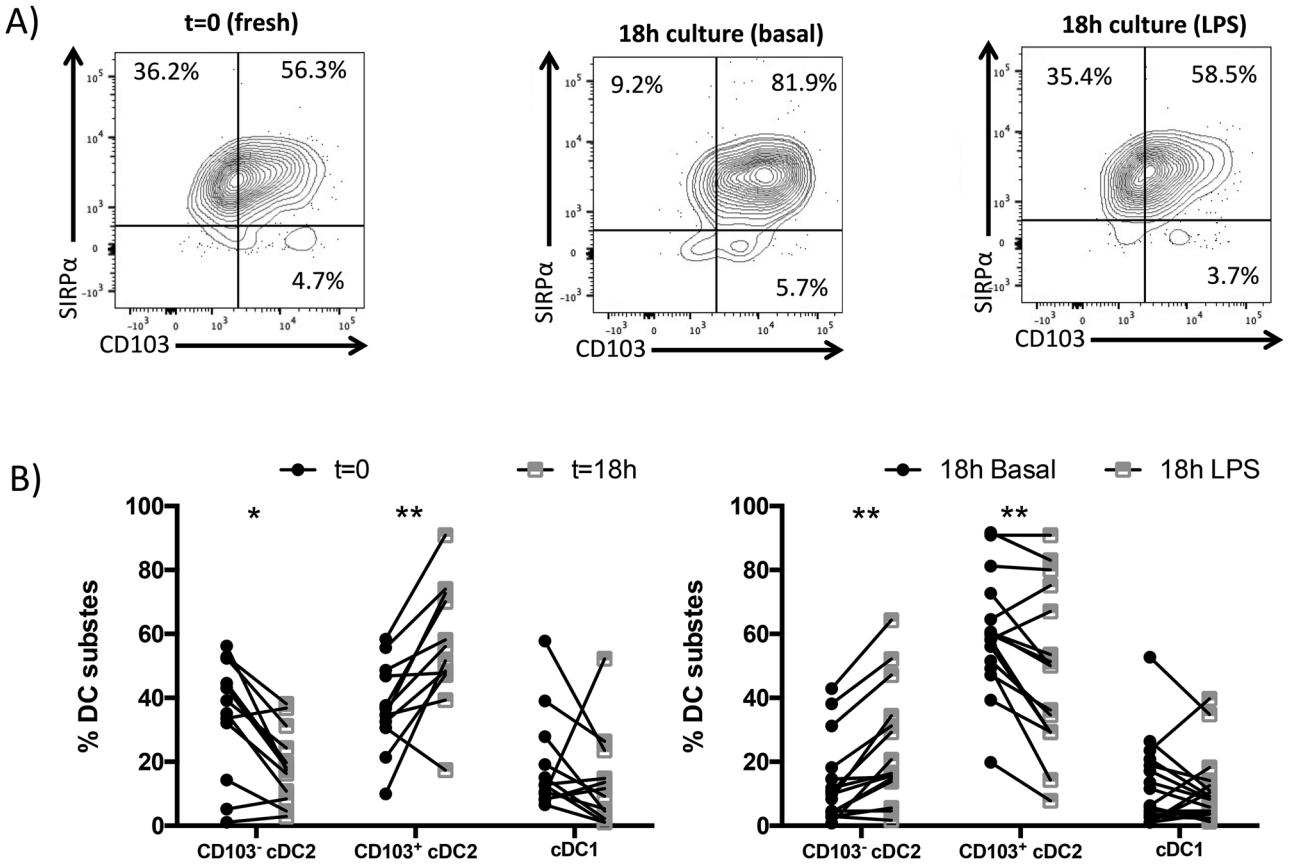

FIGURE 3 | CD103$^+$ conventional dendritic cells increase their proportion following culture. (A) Human intestinal conventional dendritic cells (cDC) were identified in Figure 1C within fresh lamina propria mononuclear cells (LPMC) as well as after overnight culture in resting conditions or in the presence of LPS. LPMC were obtained from colonic biopsies from controls. Pooled results from several independent experiments are shown in (B). Two-way ANOVA repeated measures with Sidak correction was applied in (B). *p*-values <0.05 were considered significant (*$p < 0.05$; **$p < 0.01$).

elicited over allogenic naïve T-cells (Figure 8A), although that did not translate into a differential cytokine profile (Figure 8B). Nevertheless, although cDC subsets from the inflamed ileum from CD patients did not display such increased stimulatory capacity (Figure 8C), which was likely due to the increased stimulatory capacity that ileal cDC already have (Figure 5E), CD103$^+$ cDC2 from such tissue had an increased capacity to generate IL-17$^+$ helper T-cells (Figure 8D).

4 | Discussion

Although the mechanisms of immune tolerance in the human gut have been traditionally related to CD103$^+$ cDC2, recent evidence suggests that cDC1 is also needed to achieve such a goal [37]. Hence, both cCD1 and cDC2 are essential to prime the mechanisms of intestinal tolerance in a retinoic acid-dependent manner [11, 12]. Indeed, the role of PD-L1 on intestinal cDC to maintain the mechanisms of immune homeostasis is more relevant in the small bowel, while in the colon, that tolerogenic effect would be XCR1-dependent [38], in agreement with the restricted PD-L1 expression that we have reported on human intestinal cDC in health. Moreover, we hereby have also proved that all ileal cDC subsets are more stimulatory than their colonic counterparts [29] at the time that have also unveiled how all human intestinal cDC subsets prime the generation of IL-10$^+$ helper T-cells, being that capacity increased in CD103$^+$ cDC (both cDC1 and cDC2) in agreement with the regulatory capacity attributed to these cells [29].

Specifically, referring to the colon from IBD patients, our results revealed that although the proportion of cDC2 was not altered in these patients, DC from IBD patients constitutively display lower levels of SIRPα regardless of IBD type (CD or UC) or mucosal condition (inflamed or noninflamed). Besides, we have also described how cDC1 and CD103$^+$ cDC2 are specifically reduced in the inflamed colon from UC patients (rendering the inflamed tissue from these patients with a higher proportion of putative CD103$^-$SIRPα^- cDC), but not in that from CD patients, describing therefore a differential immune signature between both conditions. This is an important observation since it has been previously reported that the number of CD103$^+$ cDC is lower in the inflamed colon from patients with IBD, irrespectively of its type (CD or UC) [23] or mucosal status [24], something that we have not confirmed in our patients. Indeed, although there was not a specific reduction of CD103$^+$ cDC in CD patients, these cells, however, were more stimulatory, even in the noninflamed tissue, at the time that they also acquired the capacity to prime the generation of IL-17$^+$ T-cells in the inflamed ileum. These are important observations given that the balance of immune responses induced by cDC can shift in the context of inflammation to promote

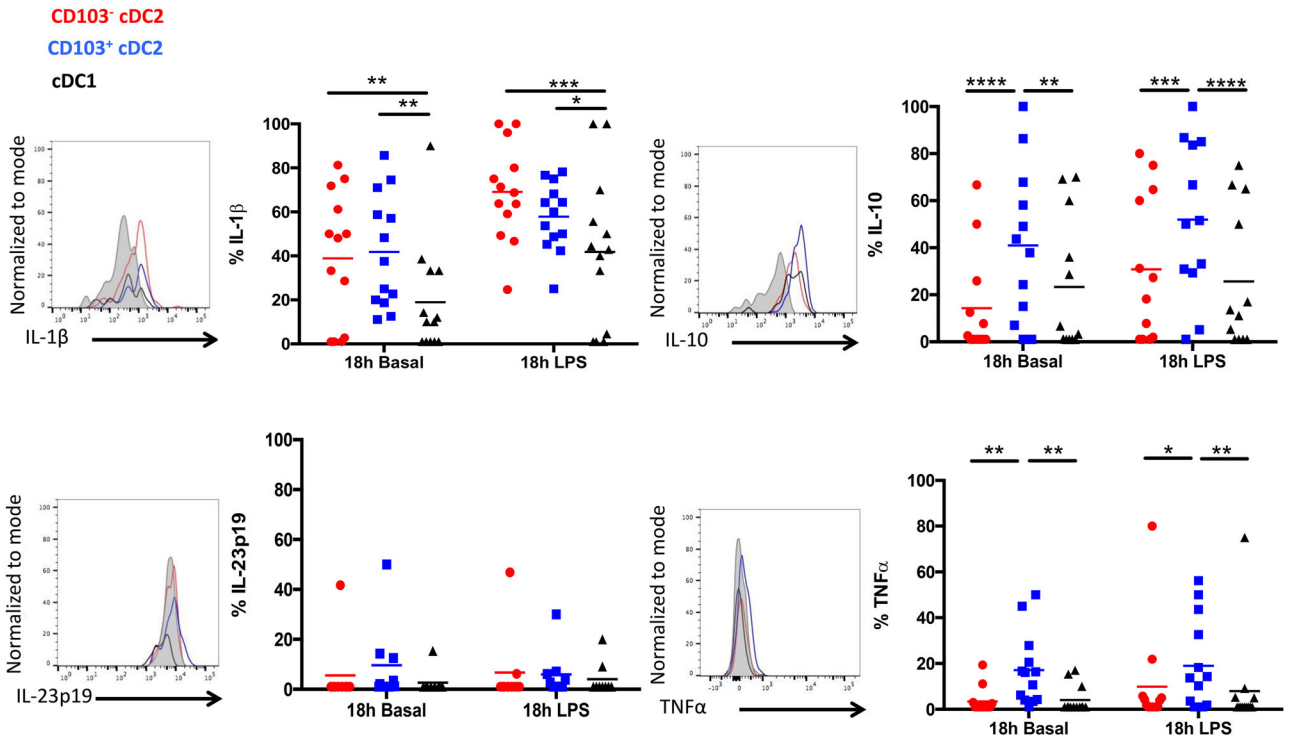

FIGURE 4 | IL-10 production is associated with CD103$^+$ type 2 conventional dendritic cells. Human colonic conventional dendritic cell (cDC) subsets were determined as in Figure 1C, and their intracellular production of IL-1β, IL-10, IL-23p19, and TNFα was determined. Histograms show representative examples of cytokine production within each given subset. The proportion of positive cells for each given marker within each cDC subset (shown on the pooled plots) was determined by the region method, referring to specific fluorescence minus one (FMO) controls (shaded histograms). Experiments were performed after 18 h of culture, both in resting conditions (basal) or in the presence of LPS, using lamina propria mononuclear cells from control biopsies. Two-way ANOVA repeated measures with Sidak correction was applied. p-values <0.05 were considered significant (*$p < 00.05$; **$p < 00.01$; ***$p < 00.001$; ****$p < 00.0001$).

effector mechanisms. In murine models, CD103$^+$ cDC signaling through the p38 MAPK pathway regulates the differentiation of naïve T cells into Treg or Th1 cells. In the absence of this signaling, retinaldehyde-specific dehydrogenase 2 (RALDH2) expression is reduced, leading to decreased Treg generation while enhancing Th1 responses [33]. In humans, RALDH activity is required on CD103$^+$ cDC for the induction of FoxP3$^+$ cells [39]. However, RALDH-expressing cDC are decreased in the colon of UC patients [23], being therefore linked to the reduction in Foxp3$^+$ Treg cell–inducing activity in patients with UC [24]. Some studies have also reported that intestinal epithelial cells (IECs) suppress the production of IL-23 and IL-12 by CD103$^+$ cDC [40]. LPMC in UC patients produced higher levels of IL-13, which facilitates the apoptosis of intestinal epithelial cells in patients with UC [41]. Consequently, the presence of fewer IECs may be linked to enhanced expression of proinflammatory cytokines in colonic CD103$^+$ cDC from UC patients [24]. These shifts in the environment influence cDC maturation to acquire proinflammatory characteristics and disrupt intestinal tolerance [33]. Indeed, different subtypes of CD are characterized by the presence of proinflammatory cytokines IL17 and IL23, which enhance Th1/Th17 responses driven by CD103$^+$ cDC [42]. Hence, our study expands those observations further, defining the altered cDC subset composition, phenotype, and function found in IBD patients, at a time that unveils specific differences between CD and UC, which may provide the tools to tailor novel cDC-focused specific targets.

Although the proportion of total cDC changes throughout the human GI-tract (Figure 1B), the relative proportion of cDC1 and cDC2 (Figure 1D) remains stable through the duodenum, terminal ileum, distal colon, and proximal colon. CD103$^+$ cDC2 are predominant in the proximal compartments of the small bowel (duodenum) as previously reported [28]. Nevertheless, although the terminal ileum is technically small bowel, given its proximity to the distal colon is not surprising that it carries a higher proportion of CD103$^-$ cDC2 as in the colon (Figure 1E). This is indeed an important consideration for human studies where the terminal ileum (and not the duodenum) is typically used to represent the small intestine. Future studies should therefore be aware that the terminal ileum may be more similar (from an immune point of view) to the colon, so it may not represent a good tissue to study the human small bowel as opposed to the duodenum.

In this regard, murine cDC subsets also have a distinct regional distribution and functional specialization along the GI-l tract, as occurs in humans. Mice cDC2 are enriched in both the colon and ileum and are characterized by CD11b$^+$SIRPα^+CD103$^-$ markers, while CD103$^+$ cDC1 predominate in the small intestine [43, 44]. These subsets also regulate different T-cell responses since murine intestinal CD103$^+$ cDC1 prime Th1 cells via IL-12 secretion, whereas their CD103$^-$ cDC2 counterparts preferentially induce Th17 and Tregs through TGF-β and retinoic acid [43, 44]. Indeed, in dextran sulfate sodium (DSS)-induced IBD models,

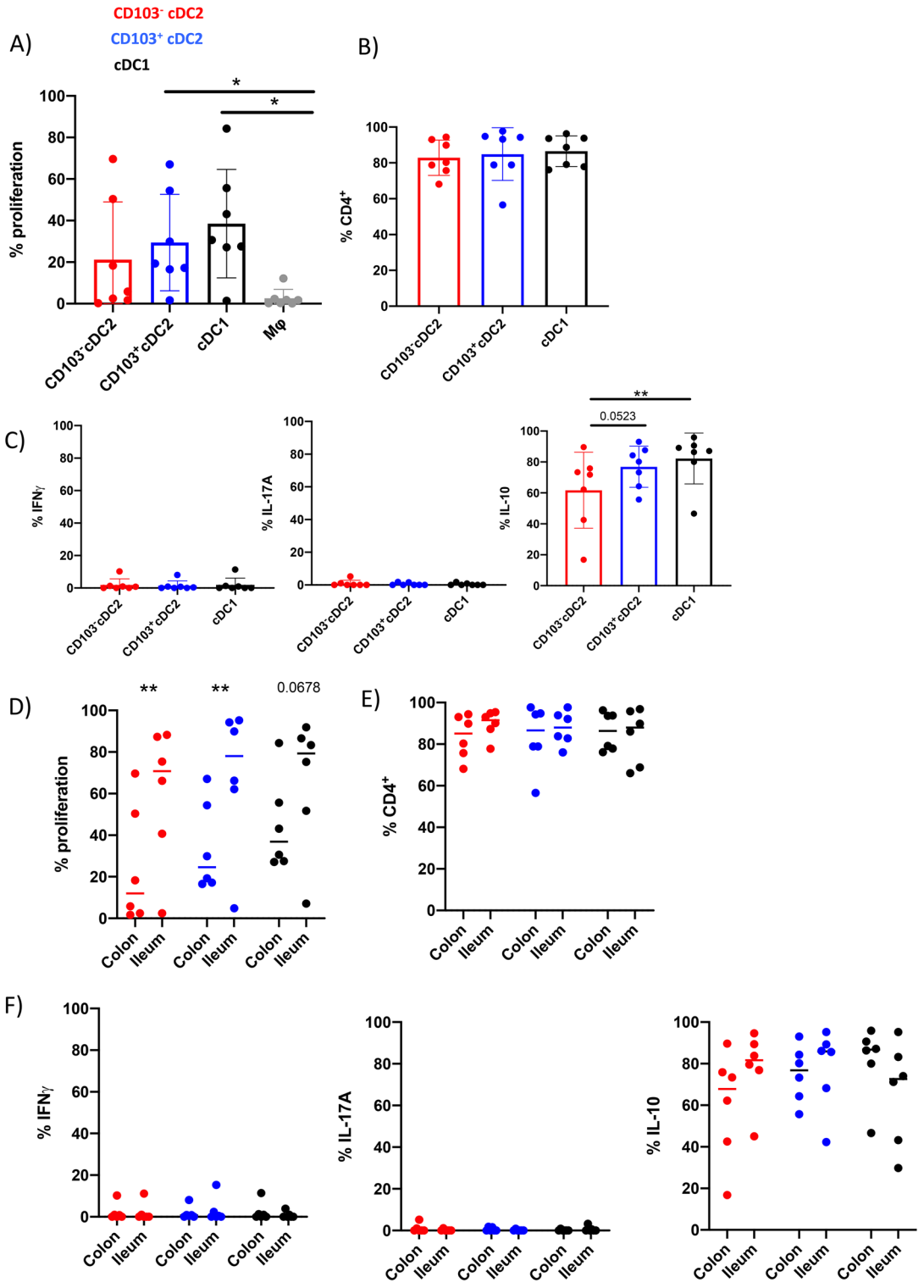

FIGURE 5 | All human intestinal conventional dendritic cell subsets prime the generation of IL-10-producing T cells. (A) Human intestinal conventional dendritic cell (cDC) subsets and macrophages from controls were sorted as in Figure S1 and used to stimulate allogenic naïve T-cells. The proportion of $CD4^+$ T-cells that had been stimulated is shown in (B), while the cytokine profile of the responding $CD4^+$ T-cells is shown in (C). (D) Comparison of the proliferation of three cDC subsets, both from the colon and the ileum; the percentage of proliferating CD4+ T-cells is shown in (E), and the cytokine profile of the responding $CD4^+$ T-cells is shown in (F). Paired one-way ANOVA with Tukey correction was applied in (A–C), while paired Two-way ANOVA with Sidak correction was applied in (D–G). *p*-values <0.05 were considered significant (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$). One-way ANOVA repeated measures with Tukey's correction was applied in all cases. *p*-values <0.05 were considered significant (*$p < 0.05$; **$p < 0.01$).

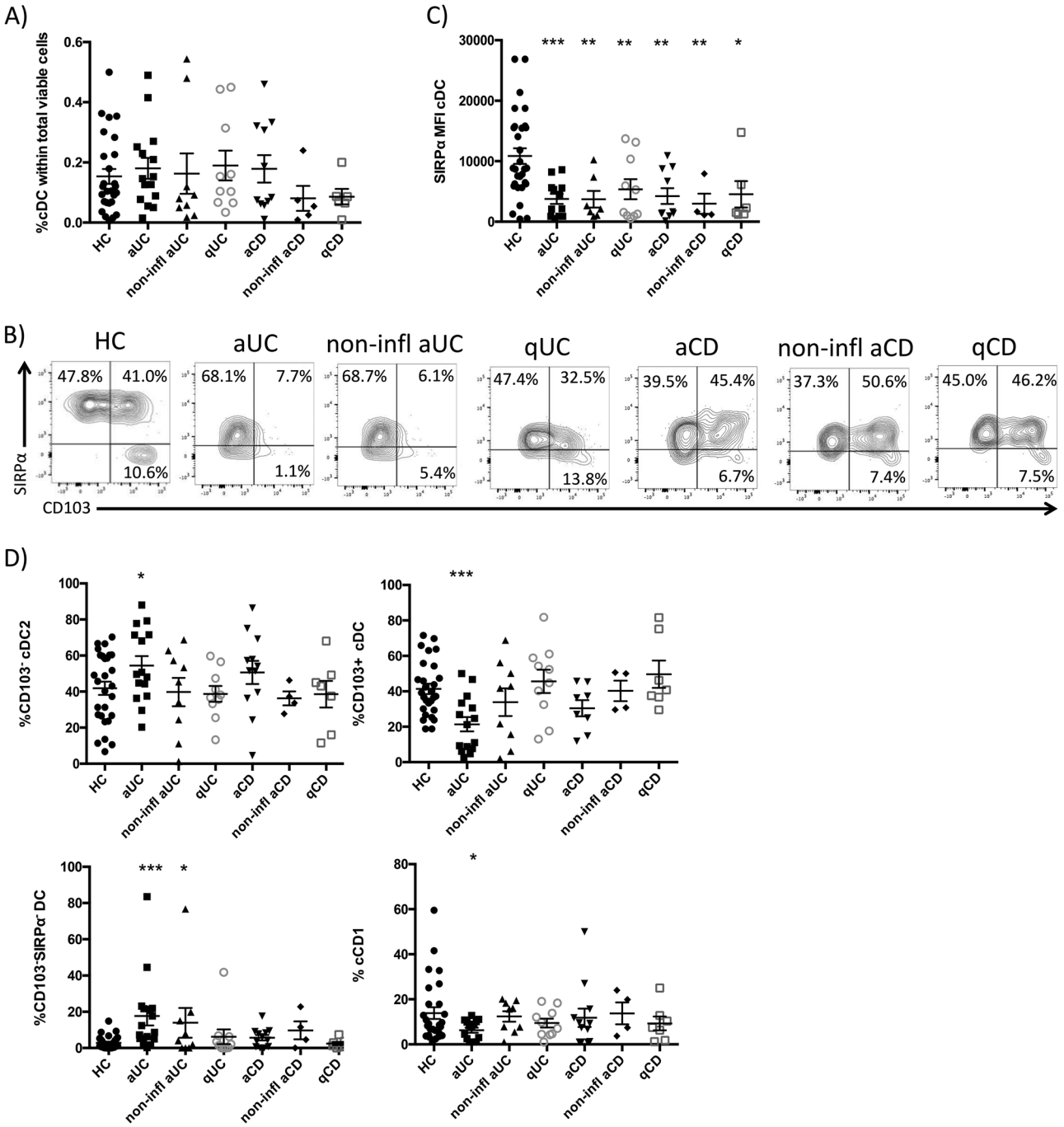

FIGURE 6 | Human intestinal conventional dendritic cells display a decreased expression of SIRPα in inflammatory bowel disease, coupled with lower numbers of type 1 and CD103$^+$ type 2 conventional dendritic cells in ulcerative colitis. (A) The proportion of human intestinal conventional dendritic cells (cDC), identified as in Figure 1A, was determined in the colon from healthy controls (HC), in the inflamed tissue from patients with active ulcerative colitis (aUC), as well as in the noninflamed colon from the same patients (non-infl aUC), and in patients with quiescent ulcerative colitis (qUC). DC proportion was also determined in the inflamed tissue from patients with active Crohn's disease (aCD) and the noninflamed tissue from the same patients (non-infl aCD), as well as in patients with quiescent disease (qCD). (B) cDC subset composition (based on the expression of CD103 and SIRPα) was also determined in the colonic mucosa from the same patients as in Figure 1C. Pooled results regarding the intensity of SIRP on total cDC, as well as the proportion of the different cDC subsets, are shown in (C) and (D), respectively. One-way ANOVA with Dunnett´s correction was applied in (C, D). Ad hoc comparisons were performed in all cases compared with the healthy mucosa. p-values <0.05 were considered significant (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

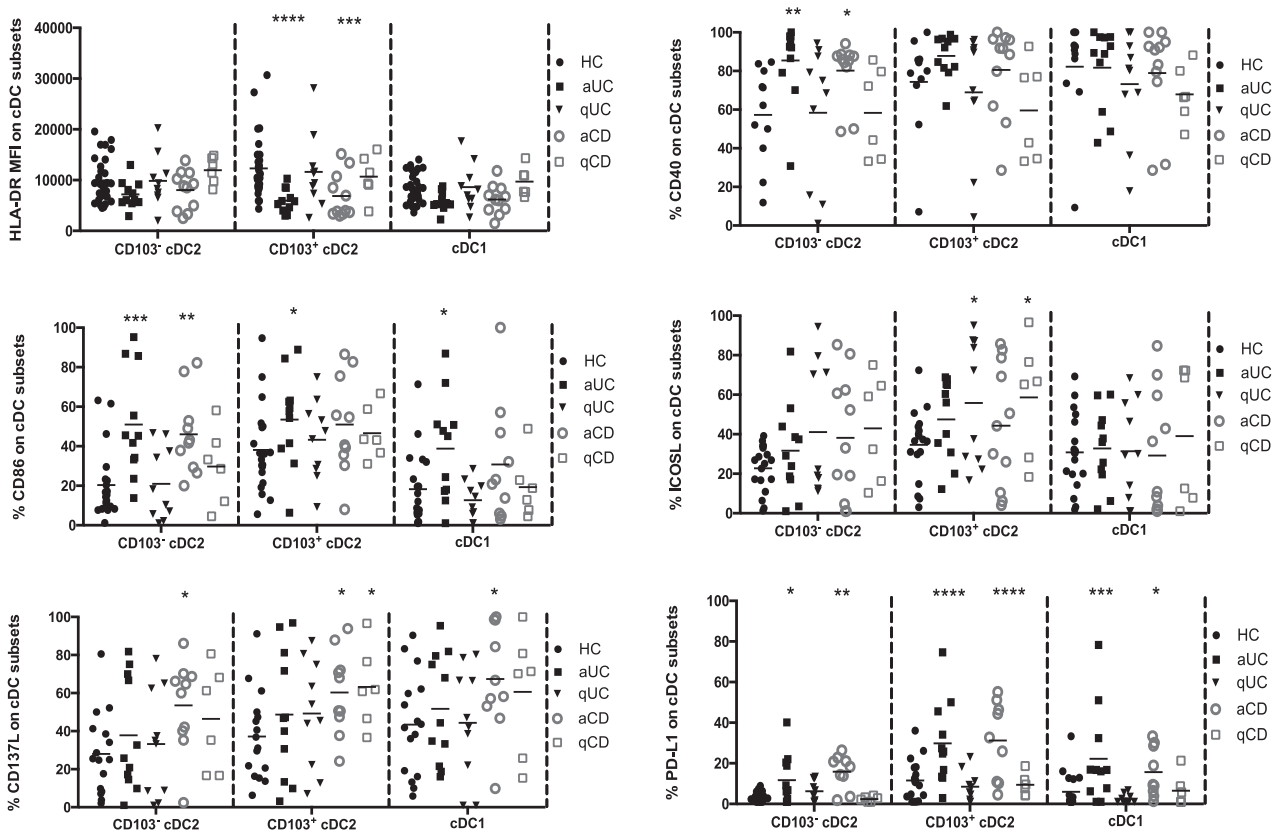

FIGURE 7 | Characterization of human conventional intestinal dendritic cell subsets in inflammatory bowel disease. Human intestinal conventional dendritic cells (cDC) subsets from colonic samples were identified as shown in Figure 1C. The expression of HLA-DR, CD40, CD86, ICOSL, CD137L, and PD-L1 on each cDC subset was further determined as in Figure 2 in the colonic mucosa from healthy controls (HC), as well as in the inflamed colon from patients with active ulcerative colitis (aUC) or Crohn´s disease (aCD), together with the noninflamed mucosa from patients with quiescent ulcerative colitis (qUC) or Crohn's disease (qCD). One-way ANOVA with Tukey correction was applied in (A), while Two-way ANOVA with Sidak correction was applied in (B). Ad hoc comparisons were performed in all cases compared with the healthy mucosa. *p*-values <0.05 were considered significant (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$).

cDC2 undergo phenotypic shifts marked by increased IL-23 production, driving pathogenic Th17 expansion while impairing Treg generation. Single-cell analyses reveal colitis-associated cDC2 clusters upregulating inflammatory genes (Il1b, Il6) and co-stimulatory molecules (CD40, CD86), correlating with enhanced T-cell activation [44, 45]. A depletion of $CD103^+CD11b^-$ DCs leads to exacerbated colitis, highlighting their role in restraining intestinal inflammation through mechanisms involving IFN-γ-induced anti-inflammatory responses in epithelial cells [46]. Together, these findings underscore the dynamic nature of cDC subsets in the gut and their pivotal roles in modulating T cell responses in the GI-tract during both tolerance and inflammatory conditions.

In agreement with the dynamic nature of intestinal cDC, it is currently unknown whether the different cDC subsets present in the mucosa truly represent different subsets or, on the contrary, different developmental or activation stages [10]. Recent evidence suggests that, at least in the mouse setting, $CD103^+$ cDC2 are derived from $CD103^-$ cDC2 counterparts following mucosal conditioning in a TGFβ-dependent manner [47]. In this regard, our results suggest that human $CD103^+$ cDC2 are likely derived from $CD103^-$ cDC2 following mucosal conditioning [47] since the proportion of $CD103^+$ cDC2 is increased following LPMC culture, although the process is prevented in the presence of proinflammatory LPS likely due to the induced proinflammatory milieu (including IL-1β) which inhibits $CD103^+$ DC differentiation in the GI-tract [48]. This would also explain the lower proportion of $CD103^+$ cDC2 found in the colon from UC patients, but not CD, as the colon from UC patients shows a more proinflammatory cytokine milieu (Figure S2), which may inhibit local differentiation of newly arrived $CD103^-$ cDC2 toward $CD103^+$ cDC2.

In agreement with that concept, circulating cDC1 and cDC2 were also studied in the blood from these patients (Figure S3A), the latter being the main subset in the human blood (Figure S3B). Indeed, both subsets displayed differences in their phenotype (Figure S3C), although the presence of IBD (either CD or UC, both active or quiescent) did not have any major impact on their phenotype (Figure S3D). Hence, these results again suggest that the altered phenotype and function of human intestinal cDC in IBD is acquired once they have entered the tissue, given that the intestinal microenvironment modulates the phenotype and function of intestinal cDC through the action of both host, dietary, and microbial-derived metabolites [13, 43, 49–51]. Hence, in the presence of a proinflammatory stimulus like the one found in the IBD mucosa intestinal cDC2, it can be reprogrammed to prime the

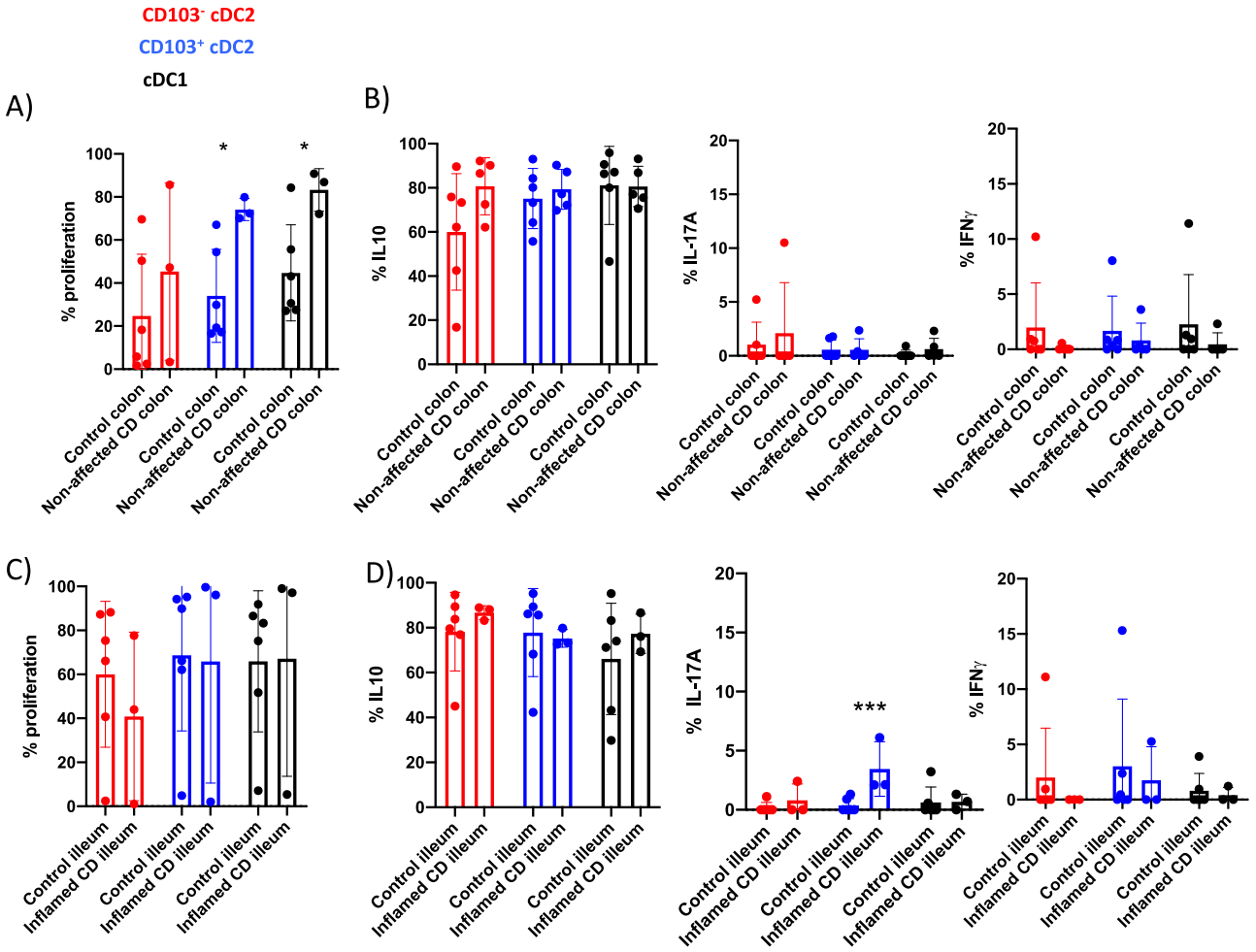

FIGURE 8 | Human intestinal conventional dendritic cells are more stimulatory in inflammatory bowel disease, and prime the generation of IL-17$^+$ T-cells in the inflamed tissue. (A) Human intestinal conventional dendritic cell (cDC) subset stimulatory capacity from the colon from controls and the noninflamed colon from patients with Crohn's disease (CD) was assessed as in Figure S1 and Figure 5, while the acquired cytokine profile of the CD4$^+$ responding T-cells is shown in (B). (C) cDC subsets stimulatory capacity and (D) the induced cytokine profile of the CD4$^+$ responding T-cells was also determined in the ileum from controls, as well as in the inflamed tissue from CD patients. Two-way ANOVA with Sidak correction was applied. *p*-values <0.05 were considered significant (*$p < 0.05$; ***$p < 0.001$).

generation of proinflammatory immune responses [52], including the generation of Th17 cells [53, 54] as in the inflamed ileum from CD patients.

SIRPα (a regulatory membrane glycoprotein) is predominantly expressed on the surface of APC, including cDC and MΦ. Its ligand, CD47, prevents cell phagocytosis by the APC on a mechanism named the "don't eat me signal". This signaling pathway has been related to cDC regulation and the development of autoimmunity [55], including murine models of IBD, where neutralization of the CD47/SIRPα signaling pathway prevents trinitrobenzene sulfonic acid-induced colitis [56]. In our work, we have described how colonic cDC from IBD patients display lower levels of SIRPα. This is in agreement with previous observations in the context of UC [24], which are hereby expanded to CD. Hence, mucosal cDC from IBD patients carry lower levels of SIRPα, irrespective of IBD type (CD or UC) or condition (inflamed or noninflamed). However, blood cDC were classified into cDC1 and cDC2 based on the expression of CD141 and CD1c, respectively (Figure S3). Hence, given that SIRPα was not included among the markers used to characterize blood cDC in IBD, we cannot confirm whether such lower expression found in the mucosa is a constitutive difference in cDC biology in IBD, or, on the contrary, an acquired phenotype once they have entered the tissue. Further studies should identify the specific mechanisms controlling the lower levels of SIRPα shown by mucosal cDC in IBD and determine its functional implications, if any, on IBD pathogenesis.

PD-L1 expression on cDC is required to suppress T-cells and, in the presence of TGFβ, to generate Treg cells [34, 35]. Within intestinal cDC, PD-L1 expression was restricted to the CD103$^+$ cDC2 subset, which also displays an enhanced capacity to produce IL-10 in agreement with the regulatory functions attributed to this subset in the maintenance of intestinal homeostasis. Nevertheless, in the inflamed IBD mucosa, PD-L1 expression was ubiquitously upregulated on all intestinal cDC subsets, confirming previous observations reporting increased PD-L1 expression within LPMC from CD patients [57] and which, together, may explain the higher numbers of FoxP3$^+$ Treg cells found in

the intestinal IBD mucosa [58]. Although this process is likely aiming to control the exacerbated immune response found in the inflamed tissue from these patients [59], we cannot discard the possibility that the PD1/PD-L1 signaling pathway is not fully functional in IBD [60].

One of the major limitations of our study is that we are aware of the large individual spread regarding cDC subset composition and phenotype in IBD patients. In this regard, it is also important to highlight that this work includes patients taking immunomodulator drugs to facilitate their recruitment (Tables S1–S4), something which can obviously affect cDC biology. Indeed, azathioprine (AZA) and mercaptopurine (6-MP) are known to impair cDC maturation and activation by downregulating costimulatory molecules and reducing proinflammatory cytokine production, promoting Treg [61]. They also decrease cDC migration, limiting their ability to activate T cells [62]. Similarly, methotrexate (MTX) suppresses cDC activation, decreases IL-12 and TNF-α levels, and enhances IL-10 production, further promoting immune tolerance [63]. Additionally, MTX can induce oxidative stress and apoptosis in DCs, reducing their viability and overall immunogenicity [63]. Nevertheless, it is also true that although these treatments can modulate cDC biology, in endoscopically active patients, they do not fully elicit their effect. However, due to the IBD clinical practice, it is also true that it is very difficult to find endoscopically active IBD patients without medication, unless they are recruited at disease onset. Given the difficulties underlying the recruitment of such type of patients in enough numbers, filling not just different IBD types (CD/UC), but also mucosal conditions (endoscopically active or quiescent), we consider that these types of studies are required to provide a translational approach as the one that we have hereby performed.

In summary, hereby have reported how human intestinal cDC can be divided into different subsets with differences in their phenotype and function, having the capacity to prime the generation of IL-10 T-cells in all of them. In addition, we have also seen that, in IBD, cDC2 display lower expression levels of SIRPα irrespective of IBD type (CD or UC) or mucosal condition (inflamed or noninflamed), while cDC1 and gut-specific CD103$^+$ cDC2 are specifically reduced in the inflamed colon from patients with UC but not with CD. This suggests the presence of different pathogenic mechanisms operating between colonic CD and UC, which may translate, therefore, into the development of better therapies that specifically target the altered routes between CD and UC.

Author Contributions

Elisa Arribas-Rodríguez performed experiments, conducted data analysis, and wrote the manuscript. Carolina G. de Castro performed experiments. Aida Fiz-López, Ángel De Prado, and Álvaro Martín-Muñoz contributed to methodology development. Luis Fernández Salazar, Jesús Barrio, Sandra Izquierdo, Javier García Alonso, Beatriz de Andrés, José María García-Abril, Alejandro Romero, Javier Sánchez González, and Cecilio Santander were responsible for patient recruitment, clinical assessment, and sample collection. Eduardo Arranz contributed to methodology and data validation. María Chaparro, José A. Garrote, and Javier P. Gisbert contributed to study design, clinical supervision, and coordination of patient sampling. David Bernardo contributed to the study design, validation, and critical manuscript revision

Acknowledgments

Nothing to report.

Funding

The research was supported by funding from the Spanish Ministry of Science (PID2023-148270OB-I00), Junta de Castilla y León (GRS 2367A/21, IR2020-1-UVA01, IR2021-UVA04), GETECCU, and the Unit of Excellence Institute of Biomedicine and Molecular Genetics, Junta de Castilla y León (CCVC8485). D.B. is also a member of the CSIC Global Health Platform (PTI Salud Global).

Ethics Statement

This study was conducted following rigorous ethical standards to ensure the integrity and reliability of the research. Ethics approval for this project was granted by the Ethics Committee at Hospital Universitario de La Princesa (Madrid, Spain) and Hospital Clínico Universitario (Valladolid, Spain). All participants provided informed consent before inclusion in the study.

Conflicts of Interest

The authors declare no conflict of interest.

Data Availability Statement

All data supporting the findings of this study are available from the corresponding author upon reasonable request.

Peer Review

The peer review history for this article is available at https://publons.com/publon/10.1002/eji.70118.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supporting File 1: eji70118-sup-0001-Tables.docx

Supporting File 2: eji70118-sup-0002-FigureS1.pptx.

Supporting File 3: eji70118-sup-0003-FigureS2.pptx.

Supporting File 4: eji70118-sup-0004-FigureS3.pptx.