Lamina Propria CD4+LAP+ Regulatory T Cells Are Increased in Active Ulcerative Colitis but Show Increased IL-17 Expression and Reduced Suppressor Activity

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Abstract

**Background:** A CD4+CD25- regulatory T cell population expressing the surface TGF-β in its latent form LAP+ [latency associated peptide] cells was proved to be protective in experimental colitis and to be suppressive of human peripheral blood [PB] T proliferation. We investigated the frequency and function of lamina propria [LP] CD4+LAP+ T cells in inflammatory bowel disease [IBD] patients.

**Methods:** Specimens from patients undergoing colonoscopy or bowel resection for IBD and colonic cancer were used as source of lamina propria mononuclear cells [LPMC]. The ulcerative colitis [UC] group was divided according to endoscopic activity evaluated with modified Baron Score. IL-17, IFN-γ, IL-10, LAP, and Foxp3 expression in CD3+CD8- [CD4] or CD3+/CD4+ gated cell population was assessed by immunofluorescence. The ability of FACS-sorted LP CD3+CD8-[CD4] LAP+CD25- to inhibit stimulated autologous PB CD3+CD8-[CD4] LAP-CD25- cells proliferation was assessed.

**Results:** LP CD4LAP+ cells were significantly increased, when compared with controls, in active UC patients and not in Crohn‘s disease patients. The majority of LP CD4+LAP+ cells were Foxp3-. The percentage of IL-17+ cells in LP CD3+CD8-[CD4] LAP+ cells was significantly higher in active UC patients when compared with controls. LP CD3+CD8-[CD4] LAP+CD25- isolated from UC patients showed reduced or no ability to inhibit autologous PB CD3+CD8-[CD4]LAP-CD25- cell proliferation when compared with controls. Removal of IL-17+ cells from LP CD3+CD8-[CD4] LAP+ cells increases their suppressive ability.

**Conclusions:** The percentage of LP CD4LAP+ cells is increased in active UC, showing reduced suppressor activity due to their increased proportion of intracellular IL-17 expression.

**Key Words:** Inflammatory bowel disease; regulatory CD4+LAP+ cells; IL-17
1. Introduction

Inflammatory bowel disease [IBD] results from an exaggerated T cell response to gut microbiota in genetically predisposed hosts. This response is not adequately counteracted by regulatory CD4+Foxp3+ T cells [Treg], although increased.1 We have recently described both in mice with experimental colitis2 and in humans with ileal pouch-anal anastomosis [IPAA] for ulcerative colitis [UC]3 the occurrence of another type of regulatory T cell in the intestinal lamina propria. This cell is a CD4+ T cell that bears surface TGF-β linked to its latency associated peptide [LAP+ cells] but does not express Foxp3. These cells are increased, in mice, in the presence of increased intestinal permeability and during the homeostatic response to a transient increase of intestinal permeability.4,5 Their in vivo regulatory activity has been proven by the fact that they contribute to the prevention of 2,4,6-Trinitrobenzenesulfonic acid [TNBS] and adoptive transfer colitis in mice.6,7 Notable characteristics of these cells are that in mice they require IL-10 and TLR2 for their in vivo expansion8,9 and they express LAP independently of Foxp3 in the presence of TGF-β.10 Initial description of their properties in humans showed that CD4+LAP+ peripheral T cells produce IL-10 and TGF-β and are expanded in vitro by polyclonal stimulation in the presence of IL-2 and IL-8.11 In the present study, we investigated the frequency and function of lamina propria CD4+LAP+ in IBD. We found that they were selectively increased in endoscopically active UC patients, showing a significant increase of IL-17 expression when compared with controls. Functional studies demonstrated that LP CD3+CD8-CD4+LAP+CD25- cells isolated from UC patients showed reduced or no ability to inhibit autologous PB CD3+CD8-CD4+LAP+CD25- cell proliferation when compared with controls. Depletion of CD3+CD8-CD4+ LAP+ CD17+ cells increases the suppressor ability of CD4 LAP+ cells.

2. Materials and Methods

2.1. Patients and tissues

2.1.1. Surgical specimens

Surgical specimens were taken from macroscopically inflamed areas of 5 ilea and 5 colons of a total of 10 patients affected by Crohn's disease, and from colon macroscopically inflamed areas of 11 patients affected by ulcerative colitis. Mucosal samples were also collected from macroscopically unaffected ileal or colonic areas [6 ileal specimens, 11 colonic specimens] of a total of 17 patients undergoing surgery for colonic cancer.

2.1.2. Biopsy specimens

Endoscopic mucosal biopsies were obtained from a total of 23 UC patients [13 males, 10 females, mean age: 48.8 ± 4.5 year] and from a total of 19 control subjects undergoing colonoscopy for colonic cancer screening and suspected functional bowel disorders in which histology confirmed the absence of inflammatory changes. Biopsies were taken from inflamed areas in UC patients with active disease, from previously inflamed areas in UC patients with endoscopic remission [anamnestic information] and in matched areas in control subjects. Diagnosis of Crohn's disease and ulcerative colitis wasascertained according to established criteria, and site and extent of the disease were confirmed by histology.

2.1.3. Patients

In UC patients, disease activity was assessed by endoscopic modified Baron Score [mBS].12 Accordingly, patients with mBS >1 were considered as active patients [12 patients] and 11 patients were considered as in remission. At time of surgery/endoscopy none of the active patients was treated with cyclosporine, azathioprine, or anti-TNF antibodies. Two UC patients in remission were receiving azathioprine. In UC patients, disease duration was 11 ± 2.5 years with no difference between active and inactive patients.

2.2. Ethical considerations

All participants provided written informed consent. Ethical approval was provided by the Istituto Superiore di Sanità’s Ethical Committee, reference: Pre-C-899/14.

2.3. Cells isolation

LPMC were isolated from freshly resected mucosa or biopsies using a previously described DDT-EDTA collagenase method.13 Briefly, strips of mucosa [6-8 g total weight] or biopsies were washed in HBBS free of calcium and magnesium [HBSS-CMF; Hyclone, Europe LTD, Cramlington, UK]. They were then incubated in HBBS-CMF containing 1 mmol/l DTT [Sigma Chemical Co., St. Louis, MO] and antibiotics [penicillin, 100 U/ml; streptomycin, 100 mg/ ml; gentamicin, 50 mg/ml; and fungizone, 2.5 mg/ml] for 15 min [surgical specimens] or 5 min [biopsies] at room temperature. After three washings in HBSS-CMF, the mucosal strips or the biopsies were chopped into pieces and incubated in HBSS-CMF containing 0.75 mmol/l EDTA, 10 mmol/l HEPES buffer, and antibiotics, three times for 45 min each [surgical specimens] or for 15 min [biopsies] at 37°C in a humid 5% CO2 atmosphere to remove epithelial cells. After two washes, the pieces were incubated for 10–13 h [surgical specimens] or 2 h [biopsies] at 37°C in a humid 5% CO2 atmosphere in complete medium [RPMI 1640 plus 10 mM HEPES buffer, 2 mM l-glutamine, 10% heat-inactivated FCS [Hyclone], and antibiotics containing 25 U/ml collagenase V [Sigma-Aldrich, Milan, Italy] and 100 µg/ml of DNase [Roche Diagnostics, Mannheim, Germany]. After incubation, the supernatant was collected and washed twice in HBBS-CMF, and LPMC were isolated using a Ficoll–Paque Plus gradient [Amersham Pharmacia Biotech, Uppsala].

2.4. Immunofluorescence studies

2.4.1. Antibodies and reagents

APC-Cy7-labelled anti-CD3, PE/Cy7-labelled anti-IL-10, and isotype-matched Ig control were obtained from Biolegend [San Diego, CA, USA]. PE-CF594-labelled anti-CD8, V450-labelled anti-IFN-γ and isotype-matched Ig control were obtained from Becton Dickinson Horizon [St José, CA, USA]. APC-labelled anti-CD3, APC-labelled anti-CD8, FITC-labelled anti-CD8, PE-labelled anti-CD25, FITC-labelled anti-CD25, FITC-labelled anti-CD4, FITC-labelled anti-CD161, isotype-matched Ig controls, and Momensin solution [Golgi StopTM], were obtained from BD Pharmingen [St José, CA, USA]. PerCP-labelled anti-LAP [TFG-B1] and isotype-matched Ig control were obtained from R&D Systems [Minneapolis, MN, USA]. APC-labelled anti-Foxp3, Foxp3 staining buffer set, PE-labelled anti-IL-17, and isotype-matched Ig control were obtained from eBioscience [San Diego, CA, USA]. LIVE/DEAD® Fixable Aqua Dead Cell Stain Kit and SYTOX® blue dead cell stain were obtained from Life Technologies [Carlsbad, CA, USA], Phorbol-12-myristate- acetate [PMA] and ionomycin were obtained from Sigma-Aldrich.
2.4.2. Immunofluorescence staining

For frequency evaluation of CD4+LAP+, represented in Figure 1, isolated LPMC were stained with anti-human-CD3, anti-human-CD4, and anti-human LAP [TGF-β1]. In preliminary experiments, we established that the percentages of LAP+ and Foxp3+ cells were not different before and after PMA-ionomycin stimulation in the presence of Golgi Stop. Therefore, for evaluation of LAP and Foxp3 expression in combination with intracellular cytokine, LPMC isolated from biopsies or surgical specimens were cultured for 4h in complete medium with PMA [50 ng/ml; Sigma-Aldrich] ionomycin [1 μg/ml; Sigma-Aldrich], and Monensin [0.66 μl/ml GolgiStop; BD Pharimingen]. After stimulation, cells were washed and labelled with LIVE/DEAD® Fixable Aqua Dead Cell Stain for 30 min. At the end of the incubation period, cells were washed and labelled with Ab against surface antigens. Due to the down-modulation of CD4 expression after PMA-ionomycin stimulation, CD8 staining was preferred. Consequently, cells were stained with anti-human-CD3, anti-human CD8, anti-human LAP [TGF-β1], and incubated for 30 min. Cells were then washed, fixed, and permeabilised with fixation/permeabilisation solution. After 40 min, cells were washed with permeabilisation buffer and labelled with anti-human IL-10, anti-human IFN-γ, anti-human IL-17, anti-human Foxp3, or isotype-matched Ig control for 30 min. Cells were then washed twice with permeabilisation buffer solution and the percentage of viable fluorescent cells was quantified using a FACSARia [BD Biosciences, Milan, Italy]. CD4+ cells were defined as CD3+CD8- cells.

Since CD161+ cell population has been reported to be increased in UC[11,12] and indirectly shown to contain sulfatide-reactive Type II NK-T cells,[12] in a subset of experiments we also evaluated CD161+ and CD161+ LAP+ cells. As previously reported,[11,12] we observed a higher percentage of CD161+ cells in UC than in control samples in CD3+CD8-[CD4] LP cells; however, we observed very few, if any, CD161+LAP+ cells in controls or in active UC samples, suggesting that the great majority of LAP+ cells are not CD161+.

2.3. Cell populations’ purification

In experiments involving cell-sorting, LPMC and PBMC were stained with anti-CD3, anti-CD8, anti-LAP [TGF-β1], and anti-CD25 antibodies and different T cell populations were sorted using a FACSARia Cell sorter [BD Biosciences]. Dead and dying cells were excluded with SYTOX® blue dead cell stain [Life Technologies]. In some experiments, LP cells were expanded by in vitro stimulation with anti-CD3/CD28 coated beads [T cell Activation/expansion Kit, Miltenyi Biotech] according to the manufacturer’s instructions. At the end of the culture period, LP CD3+CD8-[CD4]LAP+CD25-IL-17- T cells were sorted after IL17-producing LAP+CD25- cells were identified using the IL-17 secretion Assay-Detection Kit [Miltenyi Biotech] according to the manufacturer's instructions. After exclusion of CD25+ cells, the percentages of Foxp3+ cells in sorted LP CD3+CD8-[CD4]LAP+CD25- cell population ranged from 1.5% to 3% and from 0.2% to 1.3% in sorted PB LAP-cells.

2.5. Suppression assay

CFSE- labelled PB CD3+CD8-[CD4]LAP+CD25- cells stimulated with aCD3aCD28 and aCD2 coated beads [ ratio 1 bead:2 cells] were cultured in round-bottom 96-well plates for 5 days in the absence or in the presence of autologous LP CD3+CD8-[CD4] LAP+CD25- cells at 2:1 [responder: regulatory ] ratio. Proliferation of PB CD3+CD8-[CD4]LAP+CD25- cells was assessed by the CFSE fading method. The ability of sorted LP CD3+CD8-[CD4] LAP+CD25- cells to inhibit PB CD3+CD8-[CD4]LAP+CD25- cell proliferation was defined as percentage suppression and was calculated by the following formula: [proliferation of PB CD3+CD8-[CD4]LAP+CD25- cells - proliferation of PB CD3+CD8-[CD4] LAP+CD25- cells co-cultured with LP cells] divided by [proliferation of PB CD3+CD8-[CD4] LAP+CD25- cells multiplied by 100. In some experiments, suppressor ability of LP CD3+CD8-[CD4]LAP+CD25-IL-17- T cells was evaluated.

2.6. Statistical analysis

Data were analysed in the GraphPad Prism statistical PC program [GraphPad Software, San Diego, CA, USA] using the Mann–Whitney U test and Spearman correlation. A level of p < 0.05 was considered statistically significant.

![Figure 1](image_url)

**Figure 1.** Frequency of lamina propria [LP] CD4+LAP+ regulatory T cells is increased in involved tissue of UC and not in involved tissue of CD patients. LPMC were isolated by enzymatic procedure from macroscopically disease-involved tissue of surgical specimens of patients with IBD undergoing intestinal resection. Control LPMC were isolated from surgical specimens of patients undergoing intestinal resection for non-inflammatory conditions. Frequency of LAP+ was evaluated by immunofluorescence in LP CD3+CD4+ gated cells. A: Frequency of LAP+ cells in controls, CD, and UC surgical specimens. In each series, a line shows the median value. B: Representative immunofluorescence histograms. Markers were fixed according to isotype controls. CD: Crohn’s Disease, UC: ulcerative colitis; LAP: latency associated peptide; LPMC, lamina propria mononuclear cells; IBD, inflammatory bowel disease.
3. Results

3.1. Lamina propria CD4+LAP+ are increased in ulcerative colitis and not in Crohn’s disease

We started our observations investigating the CD4+LAP+ cells frequency in LPMCs isolated from severely inflamed surgical specimens of patients with UC, Crohn’s disease (CD), and controls. As shown in Figure 1, frequencies of LP CD4+LAP+ cells were selectively increased above control values in LPMC isolated from surgical UC specimens and not in LPMC isolated from ileal or colonic surgical CD specimens.

3.2. LP CD4+LAP+ are increased in active UC and are predominantly Foxp3 negative

We then analysed the CD4+LAP+ cells frequencies in LPMCs from biopsy specimens from UC patients showing endoscopically active and inactive disease assessed with the modified Baron score. As shown in Figure 2 [panels A and B], LPMC isolated from patients with active endoscopic disease showed a significant increase in the frequency of LP CD4+LAP+ as well as CD4+Foxp3+ cells when compared with LPMC isolated from inactive patients and controls. To establish the possible overlap between the two populations, we assessed the frequency of CD4+LAP+Foxp3+ cells. The percentage of cells expressing both markers was very low, with a significant increase in active UC patients when compared with patients with endoscopic remission and controls [Figure 2, panels B and C]. However, the percentage of Foxp3+ cells in the CD4+LAP+ cell population was not different between controls and UC patients [Figure 2, panel D], confirming previous reports showing that the majority of CD4+LAP+ cells in humans and mice are indeed Foxp3+. Similarly, the percentage of LAP+ cells in LP CD4+Foxp3+ cells did not show significant changes between controls and patients (controls: 5.7 ± 2.2; UC remission: 5.2 ± 2.39; UC active: 7.0 ± 1.7; mean ± standard error of the mean [SE]).

3.3. LP CD4+ LAP+ cells are regulatory cells in control patients, but not in active UC patients

As CD4+LAP+ cells were reported to show suppressor activity, we tested the suppressor activity of sorted LP CD3+CD8- [CD4] LAP+CD25- cells isolated from surgical specimens of controls and active UC patients. Suppressor activity of LP CD3+CD8- [CD4] LAP+CD25- cells was tested as the ability to inhibit proliferation of autologous PB CD3+CD8- [CD4] LAP+CD25- cells stimulated with anti-CD3, anti-CD2 and anti-CD28 mAb-coated beads. As shown in Figure 3, we found that whereas LP CD3+CD8- [CD4] LAP+CD25- cells obtained from control specimens showed the ability to suppress PB CD3+CD8- [CD4] LAP+CD25- cell proliferation, LP CD3+CD8- [CD4] LAP+CD25- cells isolated from four out of five UC patients did not inhibit the proliferation of autologous PB CD3+CD8- [CD4] LAP+CD25- cells.

3.4. LP CD4+LAP+ cells express IL-17, IL-10, and IFN-γ, and are enriched for IL-17 expression in active UC patients

To get insight into the nature of LP CD4+LAP+ cells, we analysed their cytokine expression by intracellular immune-fluorescence staining. We observed [Figure 4] a significant increase of the percentage

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Figure 2. Lamina propria CD3+CD8- [CD4] LAP+ cells are increased in active UC and are predominantly Foxp3 negative. LPMC were isolated by enzymatic procedure from endoscopic biopsies, stained, and analysed as described in the Methods section. Panel A: Percentage of LAP+ and Foxp3+ cells in LP CD3+CD8-[CD4] gated cells isolated from biopsies from control subjects, UC patients with active disease, and UC patients in remission. Panel B: Representative dot plots of Foxp3 and LAP expression in LP CD3+CD8-[CD4] gated cells. Markers were fixed according to isotype controls. Panel C: Percentage of LAP+Foxp3+ cells in LP CD3+CD8-[CD4] gated cells. Panel D: Percentage of Foxp3+ cells in CD3+CD8-[CD4] LAP+ cells. In each series, a line shows the median value. UC, ulcerative colitis; LAP, latency associated peptide; LPMC, lamina propria mononuclear cells.
of IL-17+CD3+CD8-[CD4]LAP+ cells in LP cells isolated from active UC patients when compared with controls [Figure 4, panel A] whereas no statistically significant difference was observed in the percentage of IL-10+ or IFN-γ+ cells [panels B and C]. Evaluation of the frequency of the assessed cytokines in the CD3+ CD8-[CD4]LAP+ cell population shows that in active UC, CD4+LAP+ cells are significantly enriched for IL-17 expression when compared with controls [Figure 4, panel D]. As also shown in Figure 4, panels AC, and consistently with previous studies,13,14 we observed a significantly increased proportion of LP CD3+ CD8- [CD4] IL-17+ and CD3+CD8-[CD4]IL-10+ cells, and a significantly reduced proportion of LP CD3+CD8-[CD4]IFN-γ+ cells in active UC patients when compared with controls.

3.5. Depletion of LP CD4+LAP+ IL17+ cells increases the suppressor activity of LP CD4+LAP+ cells

Since proportion of IL-17 expression was significantly increased in the LP CD4+LAP+ cells of UC patients, we investigated whether the reduced suppressive capacity of CD4+LAP+ cells isolated from patients with active disease was somehow linked to the expression of IL-17 in LAP+ cells. As shown in Figure 5 [panel A], we found that suppressor activity of LP CD4+LAP+ cells inversely correlates with the proportion of CD4+LAP+ cells expressing IL-17 [Figure 5A]. In the attempt to elucidate the contribution of IL-17 expression to the CD4+LAP+ suppressor activity, we in vitro increased the number of LP cells [see Methods] in order to obtain enough cells to test the suppressor ability of CD4+LAP+ cells in the presence or in the absence of CD4+LAP+ IL-17+ cells [see Methods]. As shown in Figure 5 [panels B and C], we observed a significantly increased suppression of PB cell proliferation by LP CD3+CD8-[CD4]LAP-depleted of IL-17+ cells when compared with LP CD3+CD8-[CD4] LAP+ undepleted cells.

4. Discussion

In the present study we demonstrated that in IBD LP CD4+LAP+ cells are significantly increased in UC and not in CD. These cells are mostly Foxp3- and co-express IL-17, IL-10, and IFN-γ both in control and in UC patients. Notably, in active UC patients LP CD4+LAP+Foxp3- cells are enriched for IL-17 expression. When isolated from LP of non-inflammatory controls, CD4+LAP+ cells show suppressor activity which is greatly reduced in LP CD4+LAP+ cells isolated from UC patients. The reduced suppressor activity is linked to their IL-17 intracellular expression, since selective depletion of LP CD4+LAP+ IL-17+ cells from the CD4+LAP+ population increases the suppressor activity of the latter cell population. This finding does not exclude the possibility that additional factors, although to a lesser extent, might influence CD4+LAP+ cells suppressor activity in UC. Reasons for the selective increase of LP CD4+LAP+ cells in UC when compared with CD may reside in the different mucosal cytokine environment associated with the two diseases favouring or not the expansion of CD4+LAP+ cells. In mice, CD4+LAP+ cell expansion has been reported to be dependent on IL-10 and TGF-β.5,15 In a previous study, we demonstrated that, in vitro, αCD3/2/28 stimulated LP T cells isolated from UC patients produced a significantly increased amount of TGF-β when compared with LP T cells isolated from CD patients that showed values comparable to controls.10 Moreover, previous studies showed a highly significant increase of IL-10 mRNA levels and an increased frequency of IL-10-positive cells in LP T cells isolated from patients...
with UC when compared with LP T cells isolated from CD patients that showed values not different from controls.  Similarly, IL-10 serum levels were significantly increased in UC active patients when compared with CD patients and controls. In the present study, confirming previous studies, we observed an increased frequency of LP CD4+IL-10-positive cells in UC, suggesting that increased TGF-β and IL-10 production may have a role in the expansion of CD4+LAP+ cells in ulcerative colitis.

The LP CD4+LAP+ cell population described in the present study is predominantly Foxp3- as previously described in human peripheral blood and in tumour-infiltrating lymphocytes in colorectal cancer, as well as in mice colonic lamina propria and airway mucosa. In animal models of experimental colitis and asthmatic lung inflammation, CD4+LAP+ Foxp3- cells have been proven to be regulatory cells. In human peripheral blood and in colonic cancer-infiltrating lymphocytes, they exhibit suppressive activity in vitro; therefore they represent a regulatory T cell subset different from Foxp3+ Treg cells also in humans.

In the present study, we observed a significant reduction of suppressive activity of LP CD4+LAP+CD25- cells isolated from inflamed UC specimens when compared with suppressor activity of LP CD4+LAP+CD25- cells isolated from control specimens, suggesting that the inflammatory milieu influences their suppressive ability. In particular, we demonstrated that in UC, inflammation is associated with a significant increase in percentage of IL-17+ cells in LP CD4+LAP+ population and that this subpopulation of CD4+LAP+ cells does not show in vitro suppressor ability. In patients with colorectal cancer, it was shown that tumour-infiltrating CD4+LAP+ lymphocytes showed a high suppressor activity, and exhibited an increase in IL-10 and a reduction of IFN-γ and IL-17 intracellular expression when compared with CD4+LAP+ in tumour-free mucosa. These data suggest that CD4+LAP+ suppressor activity was mostly associated with intracellular IL-10 expression although, in the reported studies, the suppressor ability of CD4+LAP+ isolated from tumour-free mucosa was not assessed. In previous studies performed on human peripheral blood, although CD4+LAP+ cells were characterised by their ability to secrete, upon activation, different cytokines [namely, IL-8, IL-9, IL-10, IFN-γ, and TGF-β], the contribution of CD4+LAP+ cell subsets expressing different cytokines to the suppression ability of the whole CD4+LAP+ cells population was not evaluated. To our knowledge, this is the first observation showing that CD4+LAP+ IL-17+ cells are not suppressor cells, since their removal increases the suppressive ability of CD4+LAP+ cells.

Whether CD4+LAP+IL-17+, CD4+LAP+IFN-γ+, and CD4+LAP+IL-10+ cells represent distinct cell populations derived from an initial CD4+LAP+ cell that acquires, as a terminally differentiated cell, the ability to produce different cytokines under the influence of tissue microenvironment, is not known. It is also presently unknown whether CD4+LAP+ cells producing different cytokines can derive from an initial CD4+LAP+IL-10+ cell with regulatory function that changes its cytokine pattern and function under the influence of the local microenvironment. Similarly, it is not known whether CD4+LAP+ cells co-express multiple cytokines. Regardless of these aspects, it is interesting to note that the type of cytokine produced
by CD4+LAP+ are all influenced by TGF-β itself, suggesting a possible autocrine mechanism of cytokine production regulation.\textsuperscript{18,19,20}\n
Further studies focused on CD4+ LAP+ cells biology are necessary to clarify these issues.

Although we did not investigate the possible co-expression of different cytokines in LAP+ cells, from our results it appears that, at least in UC, the expression of IL-17 in CD4+LAP+ cells reduces the overall ability of CD4LAP+ cells to inhibit autologous cell proliferation independently of concomitant co-expression of other cytokines in the CD4LAP+IL-17+ cells. The reduced suppressor activity of CD4LAP+ regulatory cells in UC reinforces the differences observed in the regulatory cell subsets between UC and CD, where a distinct CD4LAP+ regulatory cells in UC reinforces the differences observed in the CD4LAP+IL-17+ cells. The reduced suppressor activity of CD4LAP+ cells in UC might have been associated with a protective role of IL-17+/Foxp3+ T cell populations. The above reported differences between CD and UC patients highlight possible different outcomes in the two diseases of therapeutic strategies aimed to specifically block Th17 polarisation and function.

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Conflict of Interest
The authors declare no conflict of interest.

Author Contributions
Study conception and design: MB. Acquisition of data: AD, A C, AA, AZ, AB, M, Mbiffoni, AP, CM, AK, RP. Analysis and interpretation of data: AD, AC, AA, AZ, AB, MS, MB, AP, CM, AK, RP, MB. Drafting of manuscript: MB. Critical revision: AD, AC, AA, AZ, AB, MS, MB, AP, CM, AK, RP. All authors gave final approval of the version to be submitted.

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