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Tofacitinib downregulates JAK1 and JAK3 on human intestinal monocytes and macrophages without affecting dendritic cells phenotype or function

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ABSTRACT

Background: Ulcerative colitis (UC) is an inflammatory disorder of the gastrointestinal tract. Although Tofacitinib, which inhibits the JAK1 and JAK3 signalling pathway, is approved to treat patients with UC, its specific mechanism of action remain elusive. Given the central role that conventional dendritic cells (cDC) elicit in gut homeostasis, we hypothesised that Tofacitinib acts modulating cDC function in UC.

Methods: Human biopsies were obtained from colon of controls, and patients with UC (active and quiescent). Lamina propria mononuclear cells (LPMC) were *ex-vivo* cultured in the presence/absence of Tofacitinib. The specific effect elicited over human intestinal cDC, monocytes and macrophages was assessed by flow cytometry. cDC were also enriched following Tofacitinib conditioning in order to assess its effect over naïve T-cells.

Results: Several human intestinal cDC, monocyte and macrophage subsets can be found in the human colon, with these cells being more similar between controls and patients with qUC referred to patients with aUC. Following *ex-vivo* culture, Tofacitinib downregulated JAK1 expression on intestinal monocytes from patients with both active and quiescent UC. As for macrophages, JAK1 was decreased on patients with active UC while JAK was downregulated on macrophages from patients with quiescent disease. Tofacitinib did not modulate the phenotype or function of human intestinal cDC.

Conclusion: Tofacitinib does not modulate the phenotype and function of human intestinal cDC in UC. On the contrary, it displays a differential capacity to modulate intestinal monocyte and macrophage phenotype. Future studies should address whether it also translates into a differential function of these cells.

1. Introduction

The gastrointestinal (GI)-tract is in contact with a wide variety of commensal microbiota and diverse pathogens. Therefore, it requires a balance between immunity and immune tolerance; the lack of immune responses, or immune tolerance, to food antigens and the commensal microbiota is essential to keep the homeostasis of the GI-tract [1]. Inflammatory bowel disease (IBD), which includes ulcerative colitis (UC) and Crohn's Disease (CD), is an inflammatory disorder of the GI-tract characterized by an uncontrolled inflammation and abnormal activation of the immune system that occurs when intestinal homeostasis becomes dysregulated [2]. Although the aetiology of IBD remains largely unknown, studies indicate that the individual's genetic susceptibility, external environment, intestinal microbiota and immune responses are all involved and functionally integrated in the pathogenesis of IBD [2,3].

While CD can cause transmural inflammation and affect any part of the GI-tract in a non-continuous manner, UC is typified by mucosal

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inflammation and limited to the colon [2]. Development of targeted gut-specific therapy for IBD is still an unmet need. UC is a serious, costly and persistent health issue with a socioeconomic impact comparable with that for other chronic diseases. Treatment involves escalating drug regimens with concomitant side effects followed by surgical interventions which are often multiple. Indeed, the most effective current available therapies like the biological drugs (antibodies targeting immune mediators like TNFα, α4β7, p40, etc.) are only effective in around 1/3 of patients so there is a need to develop novel and better compounds to treat IBD patients.

The Janus kinase (JAK) family includes four intracellular tyrosine kinases: JAK1, JAK2, JAK3, and one non-receptor tyrosine-protein kinase 2 (TYK2). These proteins associate with the intracellular portion of cytokine or hormone receptors and activate signal transducers and activators of transcription (STATs) through autophosphorylation in an intracellular signal transduction pathway [4]. Upon binding of a cytokine or hormone to its receptor, the subunits of receptors form multimers, enabling JAK proteins to phosphorylate the associated cytokine receptor. Phosphorylated intracellular cytokine receptor facilitates recruitment of STATs. JAK proteins phosphorylate STAT proteins, leading to STAT homo-dimerization. The STAT homodimer localizes to the nucleus and activates downstream transcription [4]. JAK-STAT pathways regulate signalling for multiple immune-relevant mediators, including type I interferon, IFN-γ, and IL-2, IL-4, IL-6, IL-7, IL-9, IL-12, IL-15, IL-21, IL-23, and IL-27 and they are implicated in the pathogenesis of inflammatory bowel diseases [4,5]. JAK signalling pathway plays therefore a critical role in mediating inflammatory immune responses. Specially, JAK3 appears to play an important role in driving lymphocyte development, proliferation, and differentiation as its signalling drives $CD4^+$ T-cell differentiation into specialized Type 1 helper T (Th1) and Type 2 [6]. Furthermore, IL-15 signalling through JAK3 serves as a survival signal for NK cells [6].

Building from that, Tofacitinib (CP-690550) is an oral, small-molecule, Janus kinase inhibitor currently used to treat patients with UC [6–8]. Tofacitinib interferes with the JAK-STAT signalling by competing with ATP for binding to the kinase domain of JAKs and inhibits JAK1, JAK2, and JAK3. In vitro studies, however, showed preferential inhibition of JAK1 and JAK3 with less effect on JAK2 [9]. Despite knowing this, it remains unknown which kind of cells are the specific target of Tofacitinib.

In this regard, antigen presenting cells (APC), including conventional dendritic cells (cDC), monocytes, and macrophages, are essential to maintain the mechanisms of immune tolerance towards nutrients and commensals, and immunity against invading pathogens [1,3]. Besides, the JAK-STAT signalling pathway play a key role modulating the phenotype and function of human intestinal APC [10–13].

Given therefore the central role displayed by the JAK-STAT signalling pathway on cDC, monocytes and macrophages, we hereby aimed to assess the specific contribution of these cells to disease progression in UC and identify the immunomodulatory effects that Tofacitinib elicits over them.

2. Materials and Methods

2.1. Patients and biological samples

Colonic intestinal biopsies were obtained from patients with UC undergoing a colonoscopy for disease diagnose and/monitoring. A total of 10 patients with active (aUC, defined by a Mayo endoscopic score $\geq$1; 70 % men, 52 ± 16 years) and 10 patients with quiescent disease (qUC, defined by a Mayo endoscopic score = 0; 60 % women, 59 ± 13 years) were included. Intestinal biopsies from 10 healthy controls (60 % women, 77 ± 8 years), referred for colonoscopy due to rectal bleeding, dyspepsia or colorectal cancer screening but with macroscopically and histologically normal mucosa, were also obtained. All samples were obtained at the Digestive Service from both Hospital Clínico Universitario and Hospital Universitario Río Hortega (both of them from Valladolid, Spain). In all cases, biopsies were preserved in Roswell Park Memorial Institute (RPMI) Medium (Sigma-Aldrich, Dorset, UK) at 4 °C, and processed immediately. Patient demographics including disease condition (active/quiescent), gender, age, Mayo endoscopic score, UCEIS and treatment is shown in Supplementary Table 1.

Ileocolonic resections were also obtained from 10 patients (60 % men, 77 ± 8 years) with proximal colon cancer at the General Surgery and Digestive System Service from Hospital Clínico Universitario (Valladolid) following written informed consent from the patients (approval code by the CEIm Area del Salud de Valladolid Este 19–1353). The non-affected tissue (minimum distance of 10 cm with the tumour) was preserved in Roswell Park Memorial Institute (RPMI) Medium (Sigma-Aldrich, Dorset, UK) at 4 °C until processed.

Peripheral blood samples were also obtained from healthy controls provided by the "Biobanco del Centro de Hemoterapia y Hemodonación de Castilla y León" (Valladolid, Spain).

2.2. Sample processing

Once in the lab, biopsies were incubated with 5 mL Hank's buffered salt solution (HBSS) (Gibco BRL, Paisley, Scotland, UK) supplemented with 1 mM Dithiothreitol (DTT) (ThermoFisher Scientific, Waltham, USA) and 1 mM Methylenediamine-tetra acetic acid (EDTA) (ThermoFisher Scientific, Waltham, USA) in an orbital shaker (30 min, 250 rpm, 37 °C). Following incubation, 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 1 mg/mL collagenase D (Roche Diagnostics GmbH, Mannheim, Germany), 20 μg/mL liberase (Roche Diagnostics GmbH, Mannheim, Germany) and 25U/mL benzonase (ThermoFisher Scientific, Bonn Germany) in an orbital shaker (three incubations, 30min each, 250 rpm, 37 °C). Following each incubation, the medium was filtered with a 100 μm strainer to obtain lamina propria mononuclear cells (LPMC) which were preserved at 4 °C until used. Remaining tissue was further digested two more times following the same approach. Following incubations, LPMC were collected in the same tube which was further centrifuged (300g, 10min, 4 °C) and resuspended in RPMI medium. Human intestinal resections were cleaned with HBSS and muscle and fat were subsequently removed using surgical scissors. Tissue was further cut it into smaller pieces and processed as above.

Peripheral blood samples were processed to obtain peripheral blood mononuclear cells (PBMC) by centrifugation over Ficoll-Paque PLUS (Amersham Biosciences, Chalfont St. Giles, UK).

2.3. Lamina propria mononuclear cells culture

Total LPMC from controls were further cultured in complete medium (AIM-V™ medium, Gibco BRL, Paisley, Scotland, UK) in resting conditions, as well as was 100 ng/ml lipopolysaccharide (LPS) (ThermoFisher Scientific, Waltham, USA) in the presence/absence of 100 nM tofacitinib citrate (active principle of Tofacitinib) (Sigma-Aldrich, Dorset, UK) with 5 % CO_2 at 37 °C. On the contrary, LPMC from patients with UC (either active or quiescent) were just cultured in the presence/absence of Tofacitinib as above. Following 18-h culture, cell-free culture supernatants were cryopreserved until further used while LPMC were harvested for flow cytometry staining.

2.4. Human intestinal cDC sorting and T-cell stimulation

Following LPMC culture from the tissue resections in resting conditions, as well as in the presence/absence of Tofacitinib following LPS stimulation, total cDC were sorted on a FACS Aria III cell sorter (BD Biosciences, New Jersey, USA) as previously published by our group [14]. On the other hand, total T-cells from control PBMC were

magnetically sorted following the manufacturer instructions (Human Pan T Cell Isolation Kit, Miltenyi Biotec, Bergisch Gladbach, Germany) while subsequent naïve T-cell enrichment was performed with the REAlease® CD62L MicroBead (Miltenyi Biotec, Bergisch Gladbach, Germany). Naïve T-cells were further stained with the proliferation marker CellTrace™ Violet (ThermoFisher Scientific, Waltham, USA).

Total cDC from each condition were used to stimulate naïve T-cells in a proportion of 5%–95 % on 96-well plaques for 5 days in AIM-V™ medium. In all cases, T-cells were also cultured in resting conditions as well as with phytohemagglutinin (PHA) 1 μg/ml. Following culture, T-cells were harvested and stained.

2.5. Flow cytometry staining

Cells were stained using viability dye Near-IR (Invitrogen, cat # 10154363) and blocking the unspecific unions with Fc-block (BD Pharmingen, cat # 564220). Supplementary Table 2 shows the specificity, clone, fluorochrome and source of the antibodies used. In all cases, cells were further washed in FACS buffer (PBS (ThermoFisher Scientific, Waltham, USA)) containing 1 mM EDTA and 0.02 % sodium azide (Sigma-Aldrich, Dorset, UK)) Intracellular staining was performing after fixation by adding intracellular antibodies with a permeabilizer Fix and Perm™ kit (ThermoFisher Scientific, California, USA). Cells were finally fixed with % Buffered Formalin (Protocol, cat # 032–059) for 10 min at 4 °C. Cell were then washed in FACS buffer before they were acquired (within 48 h).

2.6. Flow cytometry analysis

In all cases, cells were acquired on a Cytek Aurora (5 laser) cytometer (Cytek, California, USA) and analyzed using OMIQ Data Science platform (© Omiq, Inc. 2022).

For the supervised analysis, total HLA-DR$^+$ cells were identified withing singlet viable CD45$^+$ cells and categorized into conventional dendritic cells (cDC), monocytes and Mϕ based on the expression levels of CD14 and CD11c as shown in Supplementary Fig. 1. Further quantification of the expression levels of each marker was determined using the fluorescence minus one approach as shown in Supplementary Fig. 2.

For the unsupervised analysis, a complementary gating strategy was applied to select all APC (monocytes, macrophages and cDC) within single viable HLA-DR$^+$CD45$^+$ following exclusion of CD14$^-$CD11c$^-$ cells as shown in Supplementary Fig. 3. Building from that, an unsupervised approach applying Uniform Manifold Approximation and Projection (UMAP) algorithm was used. Subsequent FlowSOM algorithm was used to find similar cell subsets and separate them into groups in an unsupervised manner. A clustered heatmap was then created using the clusters obtained in the previous point. The refine results of FlowSOM algorithm were mapped on the UMAP in order to observe their distribution. Finally, Volcano plots were constructed with the edgeR algorithm comparing cluster differences.

2.7. Statistical analysis

For statistical analysis, GraphPad Prism 9 was used for the supervised analysis. One-Way ANOVA, and *t*-test comparisons were also applied as detailed in the Figure Legends. As for the flow cytometry unsupervised analysis, volcano plots were constructed with the edgeR algorithm comparing cluster differences in the unsupervised analysis. In all cases, a p-value under 0.05 were considered statistically significant.

3. Results

3.1. Unsupervised characterization of human colonic APC in health and UC

First of all, total human intestinal APC from the controls, as well as from patients with active and quiescent UC were identified as in Supplementary Fig. 3. UMAP analysis identified 4 major islands (Fig. 1A). The relative contribution of each marker on the UMAP structure is shown in Fig. 1B.

Given that CD11c can be used as a surrogate marker to discriminate human intestinal monocytes (CD11c$^+$) and Mϕ (CD11c$^-$) [15], monocytes and cDC seem to be restricted to island found on the top at the right as it is CD11c$^+$. Indeed, cDC seem to be found on the upper side of such island as cells are CD14$^-$ and express CD103, JAK1 and TLR2. On the contrary, the lower side of such island seem to be CD14$^+$ inactivated monocytes. On the other hand, both islands on the left seem to be Mϕ (CD11c$^-$). Although the top one express higher CD14 expression, both of them express TLR4, JAK3 and IL-1β. On the other hand, the lower island on the right seem to be non-activated JAK1$^+$ macrophages which do not express TLR4 and have lower production of IL-1β.

To further refine our analysis, FlowSOM algorithm was used to find similar cell subsets and separate them into clusters in an unsupervised manner. A total of 16 clusters were identified according to the expression of the different markers as shown in the heatmap (Fig. 1), which also reveals a close relationship between the cells from patients with quiescent UC and controls, referred to patients with active disease. These clusters were further plotted in the UMAP to relate one with each other (Fig. 1D).

Volcano plots revealed that 6 of the clusters were significantly increased in controls when compared with aUC, and only one was increased in aUC. When comparing aUC and qUC, 6 of the clusters were significantly increased in qUC and 2 of them were decreased. Finally, only 1 cluster was differentially represented between controls and patients with qUC (Fig. 1E).

3.2. Tofacitinib JAK1 down regulation is restricted to intestinal monocytes

Having described the phenotype in resting conditions of the main APC found in the human colon, we next assessed the effect that Tofacitinib elicits on these cells following LPS stimulation. Our results revealed that Tofacitinib decreased IL-6 production, as well as the expression of TLR4 and JAK3 on macrophages. On the other hand, Tofacitinib decreased JAK3 levels on monocytes while it restored CXCR3 downregulation caused by LPS on cDC (Fig. 2).

When focused on patients with active UC, our results shown that only JAK1 expression on both macrophages and monocytes, but not cDC, were decreased following Tofacitinib stimulation (Fig. 3). Similar observations were found in the case of patients with quiescent disease, where Tofacitinib decreased JAK1 and JAK3 levels, and increased TLR2 levels on monocytes, and decreased IL-15 production by cDC with no effect on macrophages (Fig. 4).

3.3. Tofacitinib does not modulate the outcome of human intestinal cDC

Although Tofacitinib did not elicit any major effect of the phenotype of human intestinal cDC from patients with UC (either active or quiescent), we finally addressed whether it could, however, modulate cDC function given their central role at driving disease inflammation in UC [1–3,16]. Given that UC patients, as opposed to those with CD hardly have tissue resections, we used a model of controls of human intestinal cDC sorted from human resection which had been previously activated with/out LPS.

Human intestinal cDC induced naïve T-cell proliferation (Supplementary Fig. 4) as opposed to the monocytes/macrophages counterparts (data not shown). Overall, our results revealed that Tofacitinib decreased the stimulatory capacity of LPS-activated colonic cDC but not their ileal counterparts (Fig. 5A). Of note, when further analysis was performed to determine whether such reduction was mainly elicited on the helper of the cytotoxic fraction, no differences were found (Fig. 5B). Finally, we also assessed the profile of the stimulated T-cells in

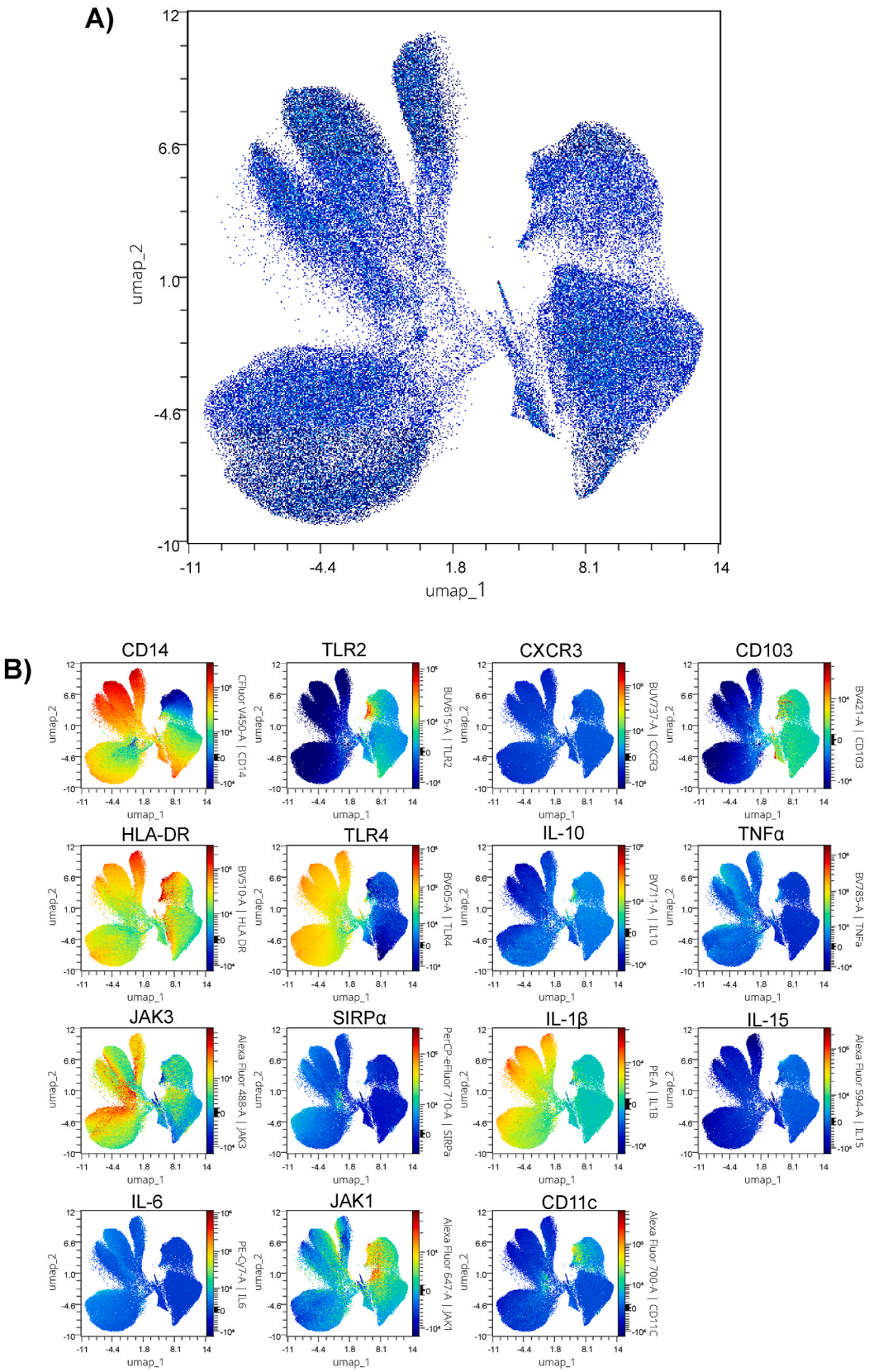

Fig. 1. Unsupervised analysis of human intestinal antigen presenting cells.
A) Total myeloid antigen presenting cells (mAPC) were identified within singlet viable leukocytes as in Supplementary Fig. 3, and analyzed with a Uniform Manifold Approximation and Projection (UMAP) on resting conditions (n = 30). **B)** Expression intensities of the analyzed markers represented with a color code based on the intensity where red represent higher expression and blue, lower expression. **C)** Heatmap displaying the intensity levels of each identified cluster within the three cohorts. **D)** All 16 clusters were overlaid on the UMAP projection using a specific color and number as shown in the legend. **E)** Volcano plots comparing the different clusters among the 3 study groups highlighting in green those with statistically significant differences.

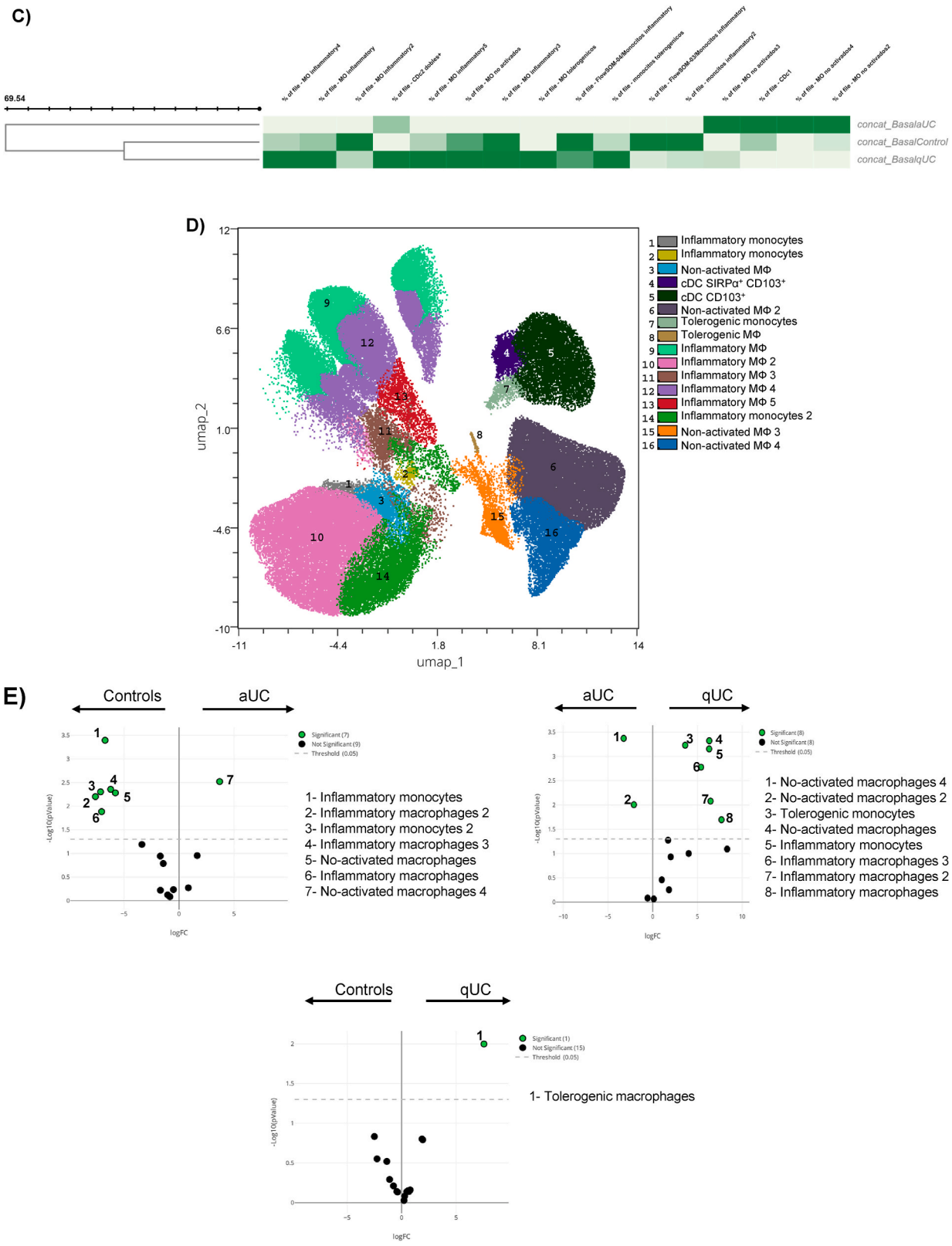

Fig. 1. (*continued*).

order to determine whether Tofacitnib could modulate the outcome of the T-cells responses towards Treg (FOXP3, IL-10), Th1 (Tbet, IFNϒ) or Th17 (RORγt, IL-17) which however was not the case (Fig. 5C) hence confirming that Tofacitinib does not modulate the phenotype and function of human intestinal cDC.

4. Discussion

Although Tofacitinib inhibits the JAK1 and JAK3 signalling pathway [9], and its use has been approved to treat patients with UC [6–8], its specific mechanism of action (i.e. its main target cell) remains elusive.

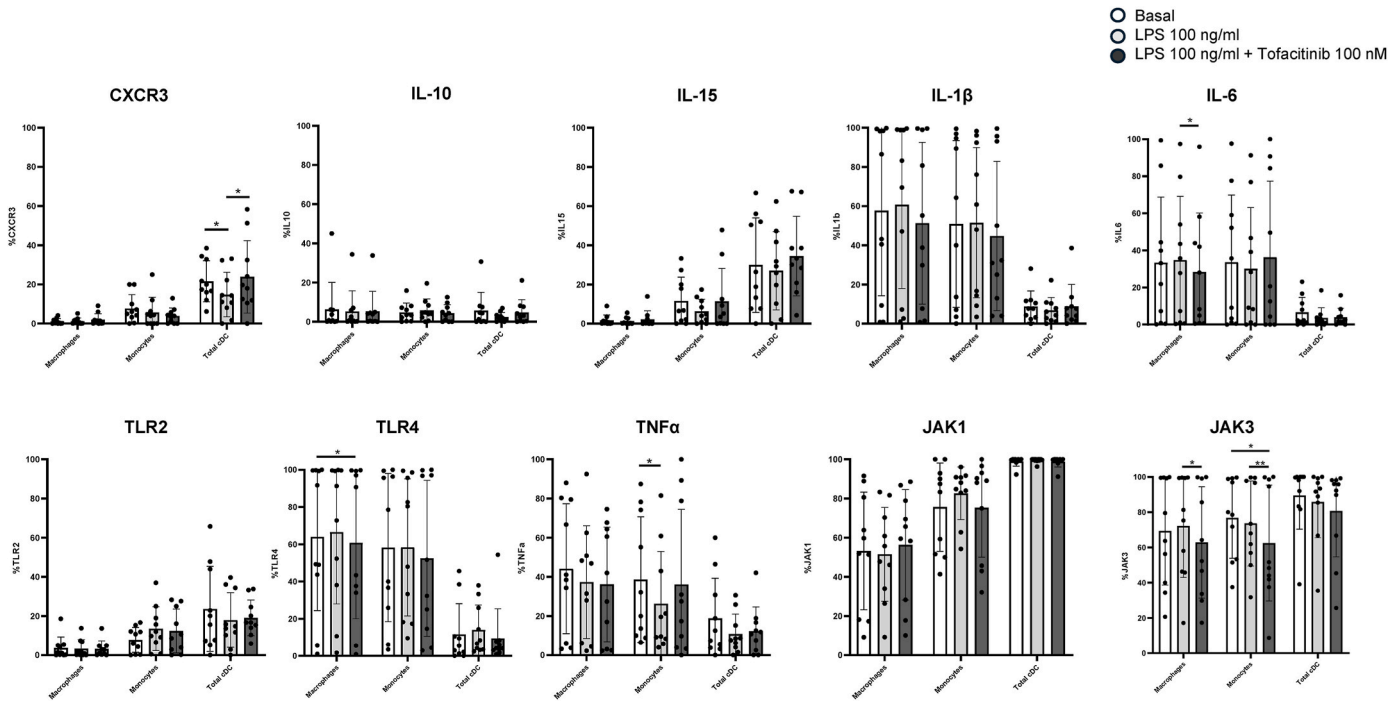

Fig. 2. Tofacitinib effect over human intestinal antigen presenting cells.
Total lamina propria mononuclear cells (LPMC) from controls, were ex-vivo cultured in resting conditions (Basal), as well as with 100 ng/ml of LPS in the presence/absence of 100 nM Tofacitinib. Total monocytes, macrophages and conventional dendritic cells (cDC) were identified as in Supplementary Fig. 1, as assessed for the expression of CXCR3, IL-10, IL-15, IL-1β, IL-6, TLR2, TLR4, TNFα, JAK1 and JAK3 based on their respective fluorescence minus one (FMO) controls as in Supplementary Fig. 2. Two-way ANOVA was applied P-values <0.05 were considered significant (*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001).

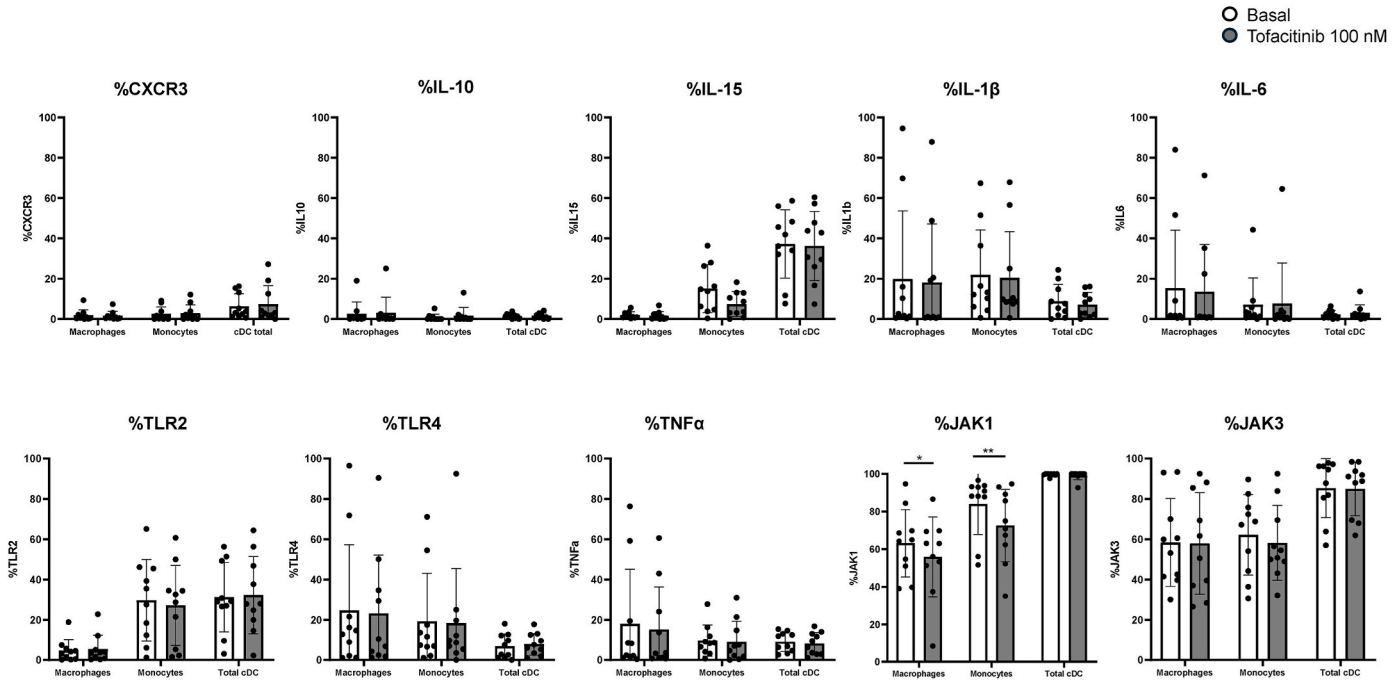

Fig. 3. Tofacitinib modulation of human intestinal antigen presenting cells from patients with active ulcerative colitis.
Total lamina propria mononuclear cells (LPMC) from patients with active ulcerative colitis were ex-vivo cultured in resting conditions (Basal) as well as with 100 nM Tofacitinib. Subsequent expression of CXCR3, IL-10, IL-15, IL-1β, IL-6, TLR2, TLR4, TNFα, JAK1 and JAK3 on monocytes, macrophages and conventional dendritic cells (cDC) was determined as in Fig. 2. Two-way ANOVA was applied panel P-values <0.05 were considered significant (*p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001).

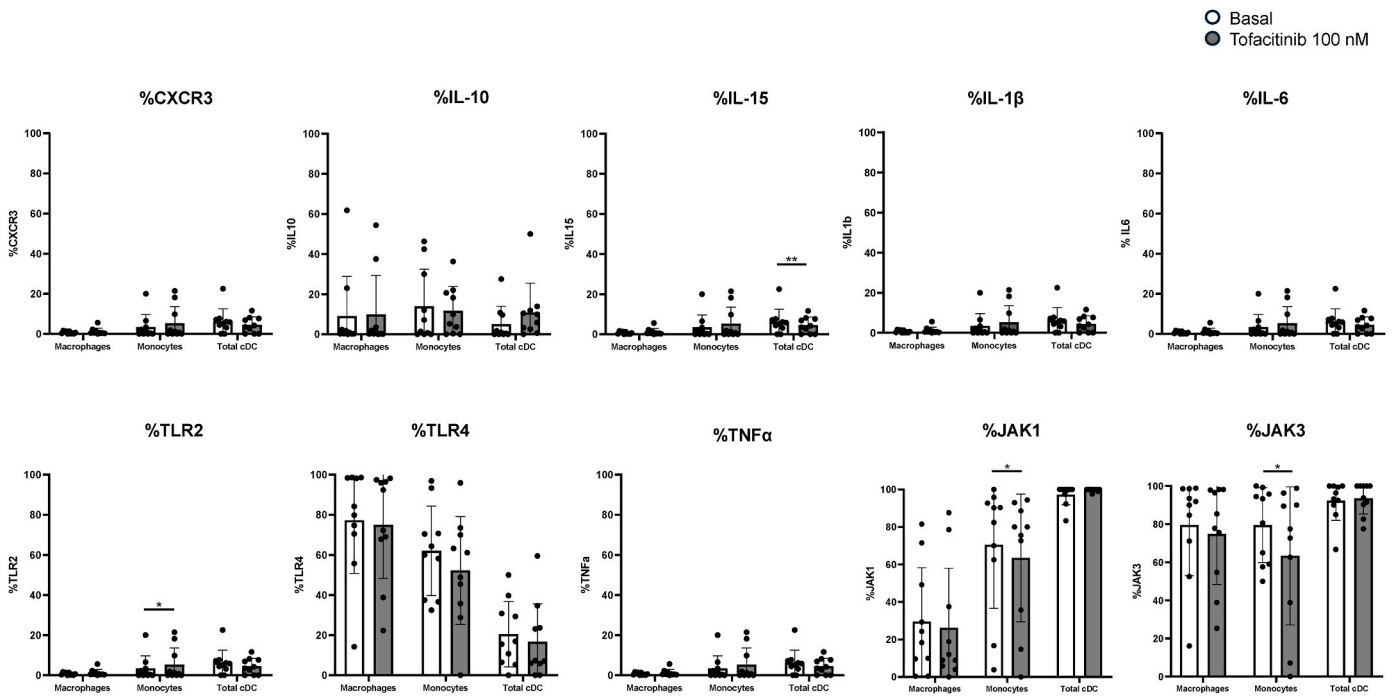

Fig. 4. Tofacitinib effect over human intestinal antigen presenting cells from patients with quiescent ulcerative colitis.
Total lamina propria mononuclear cells (LPMC) from patients with quiescent ulcerative colitis were ex-vivo cultured in resting conditions (Basal) as well as with 100 nM Tofacitinib. Subsequent expression of CXCR3, IL-10, IL-15, IL-1β, IL-6, TLR2, TLR4, TNFα, JAK1 and JAK3 on monocytes, macrophages and conventional dendritic cells (cDC) was determined as in Fig. 2. Two-way ANOVA was applied P-values <0.05 were considered significant (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$).

Given the central role that human intestinal cDC elicit controlling the outcome of immune responses in health [17], and since their phenotype and function is altered in IBD, including UC [18,19], our hypothesis was that Tofacitinib would specifically modulate their phenotype and function. In order to address such hypothesis, we assessed Tofacitinib effect over human intestinal cDC, both in health and UC, referred to human intestinal monocytes and macrophages. Nevertheless, and contrary to our expectations, we found that Tofacitinib downregulates JAK1 expression on human intestinal monocytes (and to a lower extent JAK1 and JAK3 on macrophages) without affecting human intestinal cDC phenotype or function.

Previous studies have reported that Tofacitinib decreased human monocyte-derived dendritic cell (MoDC) stimulatory capacity [20] and differentiation [21]. Nevertheless, the same has not been mirrored on human intestinal cDC. The origin of such discrepancy can be due to several reasons. The most obvious one is that those experiments were performed on LPS-activated MoDC which, nevertheless, do not resemble the properties of real cDC [22]. Besides, we hereby have performed the experiments on real human intestinal cDC so although we cannot discard that Tofacitinib may modulate the phenotype and function of circulating cDC (either in health or UC), the same does not seem to be true in the human intestinal mucosa as we have observed. In a similar manner, our observations suggest that although cDC are central to control the outcome of human adaptive immune responses, once the pro-inflammatory Th1/Th17 signalling pathway has been triggered on the UC intestine [23]Tofacitinib does not modulate the outcome of cDC. On the contrary, its therapeutic effect may be elicited suppressing pro-inflammatory T-cell activation in the human gut [24,25] without modulating cDC.

In order to further confirm Tofacitinib inhibitory in our ex-vivo model, in addition to specifically address the intracellular expression of JAK1 and JAK3, further downstream analysis revealed that Tofacinitib induced a specific reduction of phosphorylated STAT5 (but not STAT6) hence confirming Tofacitinib-induced JAK inhibition (Supplementary Fig. 5). Building from that, we hereby have found that although Tofacinitib did not seem to elicit any major effect on human intestinal cDC, it actually downregulated JAK1 on human intestinal monocytes from UC (both active and quiescent). On the contrary, JAK1 was decreased on macrophages from patients with active UC, while JAK3 was downregulated on patients with quiescent disease following Tofacitinib exposure. Previous observations have proved that Tofacitinib induces as regulatory phenotype on human primary monocytes and monocyte-derived macrophages [26–29]. Building from that, and as opposed to the previous observations on the cDC context, we hereby have confirmed, for the first time to our knowledge, that Tofacitinib downregulates JAK1 and JAK3 on *bona fide* human intestinal monocytes and macrophages.

We have also performed an unbiased characterization (in resting conditions) of the total mAPC subsets than can be found in the human colon in health and UC, both active and quiescent, by spectral and computational cytometry [30]. Our results have found that, based on the markers that we have used, we can identify up to 16 different subsets of human intestinal mAPC, being most of them macrophages in agreement with previous observations from our group [15]. Besides, and as expected, mAPC from patients with qUC were more similar to those from controls rather than to those from UC patients with active mucosal inflammation. Nevertheless, when the same approach was performed to assess whether Tofacitinib would expand or decrease a given cell cluster in any of the patients, no differences were found. Hence, these results imply that although Tofacitinib downregulated the JAK1, and to a lesser extent, the JAK3 signalling pathway on monocytes and macrophages, that is not translated into a major phenotype change on these cells. Nevertheless, whether that translates into a differential function on such cells remains elusive. Hence, a major limitation of these study is that we had focused on specifically assessing the effect that Tofacitinib elicited over cDC rather that over monocytes or macrophages. Therefore, future studies should specifically address whether Tofacitinib modulates monocytes and macrophage function or whether, on the contrary, its

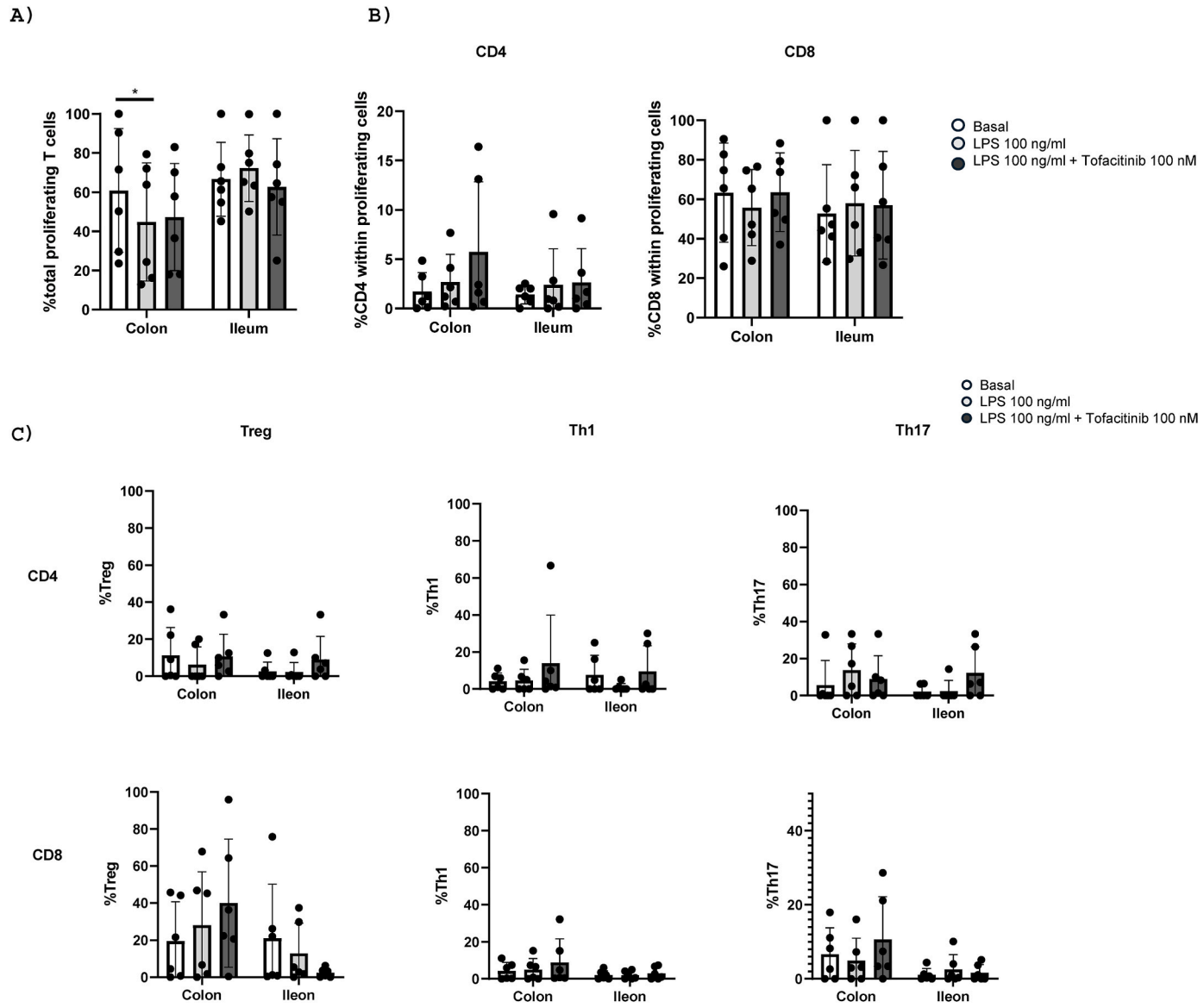

Fig. 5. Tofacitinib effect over the immunostimulatory capacity of human intestinal conventional dendritic cells.
A) Total lamina propria mononuclear cells were ex-vivo cultured in resting conditions (Basal), as well as with 100 ng/ml of LPS in the presence/absence of 100 nM Tofacitinib. Total conventional dendritic cells (cDC) were subsequently sorted and co-cultured with allogeneic cell-trace violet labelled naïve T-cells. cDC stimulatory capacity over total T-cells was determined, as well as **B)** the stimulatory capacity specifically elicited over both CD4 and CD8. The acquired phenotype (Treg: $FOXP3^{+}IL\text{-}10^{+}$; Th1: $Tbet^{+}IFN\gamma^{+}$; Th17: $ROR\gamma t^{+}IL17^{+}$ is displayed in **C)**. Two-way ANOVA was applied in all cases. P-values <0.05 were considered significant (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$).

main mechanism of action is elicited over immune cells (like T-cells as previously comments).

5. Conclusion

In summary, and although other models have suggested that Tofacitinib modifies the phenotype and function of human MoDC, we hereby have proved that human intestinal cDC are not modulated by such compound. These therefore has major implications given the differences between the mucosal and circulating immune system. Our results therefore highlight the relevance of performing experiments on the human intestinal mucosa if we want to unveil the mechanism of action of a given drug in the IBD setting given the differences between the mucosal and the circulating immune system.

CRediT authorship contribution statement

Elisa Arribas-Rodríguez: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Ángel De Prado:** Methodology, Investigation. **Beatriz de Andrés:** Resources, Conceptualization. **Benito Velayos:** Resources, Conceptualization. **Jesús Barrio:** Resources, Conceptualization. **Alejandro Romero:** Resources, Conceptualization. **Francisco Javier García-Alonso:** Resources, Conceptualization. **Álvaro Martín-Muñoz:** Methodology. **José A. Garrote:** Visualization, Validation, Resources, Investigation. **Eduardo Arranz:** Validation, Supervision, Project administration, Conceptualization. **Luis Fernández-Salazar:** Visualization, Resources, Investigation, Funding acquisition. **David Bernardo:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization.

Ethical considerations

All samples were obtained following written informed consent from the patients (PI_19–1351 CEIm Area del Salud de Valladolid Este).

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtauto.2025.100271.

Data availability

Data will be made available on request.

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