Different Intraepithelial CD3(+) Cell Numbers in Crohns Disease and Ulcerative Colitis

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Title: Different intraepithelial CD3⁺ cell numbers in Crohn's disease and ulcerative colitis

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Keywords: Diagnosis, flow cytometry, IBD, mucosal immunology.

27 **Summary:** Intraepithelial immune response can be studied by flow cytometry. The
28 proportion of CD3+ cells differs between Crohn's disease and ulcerative colitis.

To the editors,

We read with interest the paper by Roosenboom *et al.* entitled “Intestinal CD103⁺CD4⁺ and CD103⁺CD8⁺ T-cell subsets in the gut of inflammatory bowel disease patients at diagnosis and during follow-up”.¹ The authors found a decreased number of mucosal CD103⁺CD4⁺ and CD103⁺CD8⁺ T-cells in both inflammatory bowel disease (IBD) types, Crohn’s disease (CD) and ulcerative colitis (UC), which recovered after clinical remission. However, CD103⁺CD4⁺ T-cells were more abundant in both CD and UC. With a similar technical approach, we aimed at characterizing the overall numbers of T-cells (CD45⁺CD3⁺) in a Spanish CD and UC patient cohort, although focusing on the intraepithelial mucosal layer. We enrolled a total of 13 CD (8 colonic and 9 ileal samples) and 14 UC (colonic samples) patients, all of them endoscopically active. CD and UC were diagnosed using current guidelines.^{2,3} In addition, 8 non-IBD patients with inflamed mucosa (6 colonic and 4 ileal samples), and 15 patients with no macroscopic or microscopic findings (11 colonic and 7 ileal samples) were included as inflamed (IC) and healthy (Hc) controls, respectively (table 1). Detailed patient diagnosis data and materials and methods can be found in the supplementary data file.

The proportion of intraepithelial CD45⁺ cells was lower in the colon of inflamed controls compared to healthy controls (figure 1A). However, this population remained unchanged in CD (in both colon and ileum) and UC (figure 1A-B) regarding to healthy controls, which is in agreement with previous evidence.⁴ On the other side, the proportion of T-cells in UC was increased, which resulted in a decreased number of CD45⁺CD3⁺ cells. Thus, the T/CD3⁺ ratio was higher in UC patients compared to CD and the healthy control groups (figure 1C). CD ileal T/CD3⁺ ratio, as in the colon, remained normal (figure 1D). These results suggests that the intraepithelial compartment in active UC might behave different from active CD. Therefore, the T-cell subsets should be studied both in the intraepithelial layer and the lamina propria compartments separately. We are aware that our cohort is small and further research is needed. Still, the difference observed in T-cell and CD3⁺

56 numbers in the intraepithelial compartment might be a useful tool for differential
57 diagnosis of UC and it would be worth to explore in depth in UC and other forms of colitis.

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Table 1 Clinical and demographic characteristics of IBD patients and controls

Variable	Hc	IC	CD	UC
Total number of subjects	15	8	13	14
No. of colonic / ileal samples	11 / 7	6 / 4	8 / 9	14 / 0
Gender, % females	60.00%	62.50%	26.67%	43.75%
Median age (range)	52 (31-71)	51 (26-83)	27 (17-48)	46 (26-79)
Median CRP levels (range)	N/A	N/A	17 (0-31)	3 (0-284)
Montreal UC extent, %	N/A	N/A	N/A	
E1: ulcerative proctitis				3 (21.43%)
E2: left-sided UC				4 (28.57%)
E3: extensive UC				7 (50.00%)
Montreal CD location, n (%)	N/A	N/A		N/A
L1: ileal			6 (46.16%)	
L2: colonic			3 (23.08%)	
L3: ilealcolonic			4 (30.76%)	
Behaviour	N/A	N/A		N/A
B1: nonstructuring, nonpenetrating			10 (76.92%)	
B2: structuring			3 (23.08%)	

CD, Crohn's disease; Hc, healthy controls; IBD, inflammatory bowel disease; IC, inflamed non-IBD controls; N/A, not applicable; UC, ulcerative colitis.

75 **Figure legends**

76 **Figure 1: Intraepithelial T-cells are increased in ulcerative colitis.** (A-B) Percentage
77 of intraepithelial CD45⁺ cells in colonic (A) and ileal (B) samples. (C-D) T-cell/CD3⁺ cell
78 ratio in intraepithelial colonic (C) and ileal (D) samples. Biopsy samples were obtained
79 from healthy controls (Hc), non-IBD inflamed controls (IC), Crohn's disease (CD), and
80 ulcerative colitis (UC) patients. Boxes represent data within the interquartile range and
81 the horizontal bar represents the population median. Statistically significant differences
82 are shown as * $p < 0.05$.

Figure 1