Profiling of Human Circulating Dendritic Cells and Monocyte Subsets Discriminates Between Type and Mucosal Status in Patients With Inflammatory Bowel Disease

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Background: Intestinal dendritic cells (DC) and macrophages drive disease progression in patients with inflammatory bowel disease (IBD). We aimed to characterize the activation and homing profile of human circulating DC and monocyte subsets in healthy control patients (CP) and IBD patients.

Methods: Eighteen CP and 64 patients with IBD were categorized by diagnoses of Crohn disease (CD) and ulcerative colitis (UC), either endoscopically active (inflamed) or quiescent. Circulating type 1 conventional DC, type 2 conventional DC, plasmacytoid DC, classical monocytes, nonclassical monocytes, and intermediate monocytes were identified by flow cytometry in each individual and characterized for the expression of 18 markers. Association between DC/monocytes and IBD risk was tested by logistic regression. Discriminant canonical analyses were performed to classify the patients in their own endoscopy category considering all markers on each subset.

Results: CCRL1, CCR3, and CCR5 expression on circulating type 1 DC; CCRL1 expression on nonclassical monocytes; and CCR9 and β7 expression on classical monocytes allowed us to discriminate among the different study groups. Indeed, the same markers (excluding β7) were also associated with IBD when all DC and monocyte subsets were considered at the same time.

Conclusions: Monitoring the phenotype of human circulating DC and monocyte subsets may provide novel tools as biomarkers for disease diagnosis (CD/UC) or mucosal status (inflamed/noninflamed) in the absence of an invasive colonoscopy.

Key Words: inflammatory bowel disease, circulating, monocytes, dendritic cells, human

INTRODUCTION

Inflammatory bowel disease (IBD), including Crohn disease (CD) and ulcerative colitis (UC), is a global disease affecting more than 1.6 million people in the United States and more than 2.2 million people in Europe.[1, 2] Although its incidence varies widely depending on the country, it is increasing rapidly probably because of the "Westernization" of lifestyles.[3] Indeed, a large multicenter study led by our center suggests that the current incidence is greater than previously described,[4] and some studies have suggested that it may affect 1 out of 125 individuals in Western countries.[5] As a chronic disease diagnosed in early life, IBD has a high prevalence that is increasing over time. Consequently, IBD costs are considerable for health care systems.[6] It has been estimated that IBD incidence will be increased in the next generation, affecting 10 million people worldwide.[6] Under this scenario it is necessary to find new strategies not just to prevent and treat IBD, but also to develop new tools that facilitate disease diagnosis and monitoring by

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Author contributions: Study design was performed by MC and DB. MC, CS, and JPG identified, recruited, and obtained the biological samples from the patients. Sample processing was performed by LOM, SFT, ACM, IMG, and MB. Data analysis was made by LOM. Manuscript was drafted by LOM, SFT, and DB. JPG and DB obtained the funds and supervised this project during all its length. All authors read and approved the final version of the study.

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TABLE 1. Demographics of Healthy CP and Patients With IBD

	HC	Active UC	Quiescent UC	Active CD	Quiescent CD
n (%)	18 (28.1)	16 (25.0)	12 (18.7)	8 (12.5)	10 (15.6)
Age, y, mean ± SD	50.1 ± 10.2	42.1 ± 14.9	50.3 ± 13.8	55.4 ± 14.2	39.2 ± 12.4

assessing the presence and severity of endoscopic inflammation by noninvasive approaches in the absence of a colonoscopy.

Chemokines and integrins, together with their receptors, mediate leukocyte trafficking to their target tissues, including inflammatory sites.[7-9] Therefore, and not surprisingly, leukocyte homing toward the intestinal mucosa contributes to the inflammatory process in IBD.[10-12] Hence, blocking leukocyte migration toward the gastrointestinal tract is an area of extensive interest in IBD[13] given that as opposed to systemic therapeutic approaches, blocking leukocyte migration involves tissue-specific immunomodulation, thus limiting potential adverse effects. Indeed, that is the mechanism of action of the already approved anti-α4β7 vedolizumab treatment for both CD and UC patients, whereas other drugs with similar mechanisms of action (eg, etrolizumab, abrilumab, PF-00547659) are being studied for implementation in IBD clinics.[14, 15]

Intestinal dendritic cells (DC) and macrophages are essential to keep the mechanisms of immune homeostasis in health. However, under inflammatory conditions they display a proinflammatory phenotype and function, hence driving IBD progression.[16-22] Both intestinal DC and macrophages are derived from their circulating DC and monocyte precursors, which can be divided into subsets based on their ontogeny and function.[23, 24] Human circulating monocytes can be divided into classical ($CD14^+CD16^-$), intermediate ($CD14^+CD16^+$), and nonclassical ($CD14^-CD16^+$) monocytes. In a similar manner, human circulating DC can be primarily divided into plasmacytoid (pDC; $CD123^+$) and myeloid or conventional DC (cDC; $CD11c^+$) DC, with the latter being further divided into type 1 (cDC1; $CD141^+$) and type 2 (cDC2; $CD1c^+$) myeloid/conventional DC. Notably, the dysregulated properties that DC and macrophages display in IBD are not restricted to the intestinal mucosa because they can also be mirrored (at least partially) on their circulating precursors.[25-28] Hence, the study of circulating DC and monocyte subsets may provide novel tools for disease monitoring.

In this context, we aimed to characterize the activation and homing profile of human circulating DC and monocyte subsets in healthy control patients (CP) and IBD patients. This work may reveal novel pathogenic mechanisms in IBD that may vary depending on its type (CD or UC) or mucosal status (active or quiescent) and may provide evidence of novel noninvasive biomarkers to predict the presence and type of intestinal inflammation in the absence of an invasive colonoscopy.

METHODS

Patients and Biological Samples

Sixty-four patients from the gastroenterology service at Hospital Universitario de La Princesa (Madrid, Spain) were recruited (25 men; 39.1%) (Table 1)]. All samples were obtained following written informed consent after ethical approval from the ethics committee at La Princesa Hospital.

In all patients with IBD, peripheral blood (10 mL) was obtained during an ileocolonoscopy performed per clinical practice for disease diagnosis or monitoring. Patients with IBD were then categorized into different groups based on the endoscopic results. Patients with UC were divided into active (aUC) or quiescent (qUC) disease based on the Mayo endoscopic subscore (aUC: >1; Supplementary Table 1; qUC: ≤1; Supplementary Table 2). In a similar manner, CD patients were divided into active (aCD) or quiescent (qCD) based on the simplified endoscopic activity score for CD (aCD: >3; Supplementary Table 3; qCD: ≤3; Supplementary Table 4). In addition, blood samples were obtained from a total of 18 noninflamed healthy CP (50% men; aged 50.1 ± 10.2 years (mean ± SD); age interval 33–68 years]. This group had been referred because of rectal bleeding, dyspepsia, or colorectal cancer screening. All of the CP had macroscopically and histologically normal mucosa.

Blood Processing

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

Antibody Labeling and Flow Cytometry

The PBMC were stained with monoclonal antibodies and characterized by flow cytometry. In all patients, a Live/Dead fixable near–intensity ratio (IR) dead cell stain kit (Molecular Probes) was added to the cells before antibody staining, thus allowing the exclusion of dead cells from the analysis. Supplementary Table 5 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 minutes following Fc block incubation (Becton Dickinson). Cells were further

TABLE 2. Association Between the Expression of Surface Markers on cDC1 Cells, Nonclassical Monocytes, and Classical Monocytes in Patients With IBD

		Active UC		Quiescent UC		Active CD		Quiescent CD	
Subset	**Marker**	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***
cDC1 cells	CCRL1	0.23 (0.08-0.66)	0.006	—	—	—	—	—	—
	CCR5	—	—	0.10 (0.01-0.83)	0.033	—	—	—	—
	CCR3	—	—	—	—	2.29 (1.11-4.75)	0.025	—	—
Nonclassical monocytes	CCRL1	0.52 (0.28-0.95)	0.03	—	—	—	—	—	—
Classical monocytes	β7	—	—	0.56 (0.34-0.90)	0.02	—	—	—	—
	CCR9	—	—	—	—	—	—	0.64 (0.46-0.89)	0.007

OR indicates odds ratio.

washed in FACS buffer, fixed with 2% paraformaldehyde in FACS buffer for 10 minutes on ice, and washed again in FACS buffer before they were stored at 4°C before acquisition on the flow cytometer. Cells were acquired on an LSR-Fortessa (BD Biosciences) and analyzed using FlowJow (version 10.1). All cells were analyzed within singlet viable cells. Positive and negative gatings were set by the fluorescence minus one (FMO) method. The median fluorescence index (MFI) for each marker was determined within each immune subset and divided by the MFI of its respective FMO within the same subset to obtain the IR.

Statistical Analysis

Patients' individual characteristics were reported as age, sex, and IBD treatments. Differences between age according to sex were tested by *t* test analysis. A χ^2 test was performed to evaluate differences between sex. Patient categories were displayed as sample size (percentage) and age (mean ± SD).

The Kolgomorov-Smirnov normality test and univariate analysis (kurtosis and skewness) were performed to test markers for normality. Variables were transformed into their logarithm to allow them to approach normality and increase their clinical interpretability. Association between markers and IBD risk was tested by logistic regression. Results were reported as an odds ratio along with their 95% confidence interval. The sample size achieved almost 70% (β = 0.31) power to detect minimal odds ratios of 0.12. Discriminant canonical analysis was performed to classify the patients in their own endoscopy category, considering 18 homing and activation markers (β7, CCR1, CCR2, CCR3, CCR5, CCR6, CCR7, CCR9, CCRL1, CD40, CD86, CD137L, CD274 (programmed death-ligand 1), CLA, CXCR1, CXCR3, ICOSL, and HLA-DR) on each subset and including only those with a negative or positive association with IBD. A *P* value <0.05 was considered as statistically significant. All analyses were performed using SAS University Edition (SAS Institute, Cary, NC) and SPSS v.15.

RESULTS

Cell Subset Identification

Human circulating DC and monocyte subsets were studied within singlet viable PBMCs. Monocytes were identified within the CD19$^-$HLA-DR$^+$CD11c$^+$ fraction and divided into classical, nonclassical, and intermediate categories based on the expression of CD14 and CD16 (Fig. 1A). The DC were identified within CD19$^-$CD14$^-$CD16$^-$HLA-DR$^+$cells and identified as pDC (CD123$^+$) or cDC (CD11c$^+$). The latter were further divided into cDC1 and cDC2 based on the expression of CD141 and CD1c (Fig. 1A). The 18 migration and activation markers noted in the previous paragraph were determined within each of the 3 DC and 3 monocyte subsets for each IBD patient and noninflamed CP. The IR for each marker within each immune subset was determined by dividing the MFI of each marker by the MFI of its respective FMO for each monocyte (Fig. 1B) and DC subset (Fig. 1C).

Monocyte and DC Subset Analyses

There were no differences among the 5 studied groups (healthy CP, aUC, qUC, aCD, and qCD) between age according to sex (men aged 46.5 ± 13.2 years and women aged 47.7 ± 14.1 years; $P = 0.7$) and not according to sex ($P = 0.08$).

Because of the extremely high kurtosis and skewness for several markers, the IRs for each marker on each subset were log-transformed to approach normality. Descriptive analysis (medians of the IRs and their interquartile ranges) for each activation and migration marker in each studied immune cell subset are shown for healthy CP (Supplementary Table 6), aUC patients (Supplementary Table 7), qUC patients (Supplementary Table 8), aCD patients (Supplementary Table 9), and qCD patients (Supplementary Table 10).

First, we performed discriminant canonical analyses of the 18 studied markers applied over all DC and monocyte subsets (Fig. 2A). Our results showed that all patients within each study group (healthy CP, aUC, qUC, aCD, and qCD) were associated with each other clustering separated from the others.

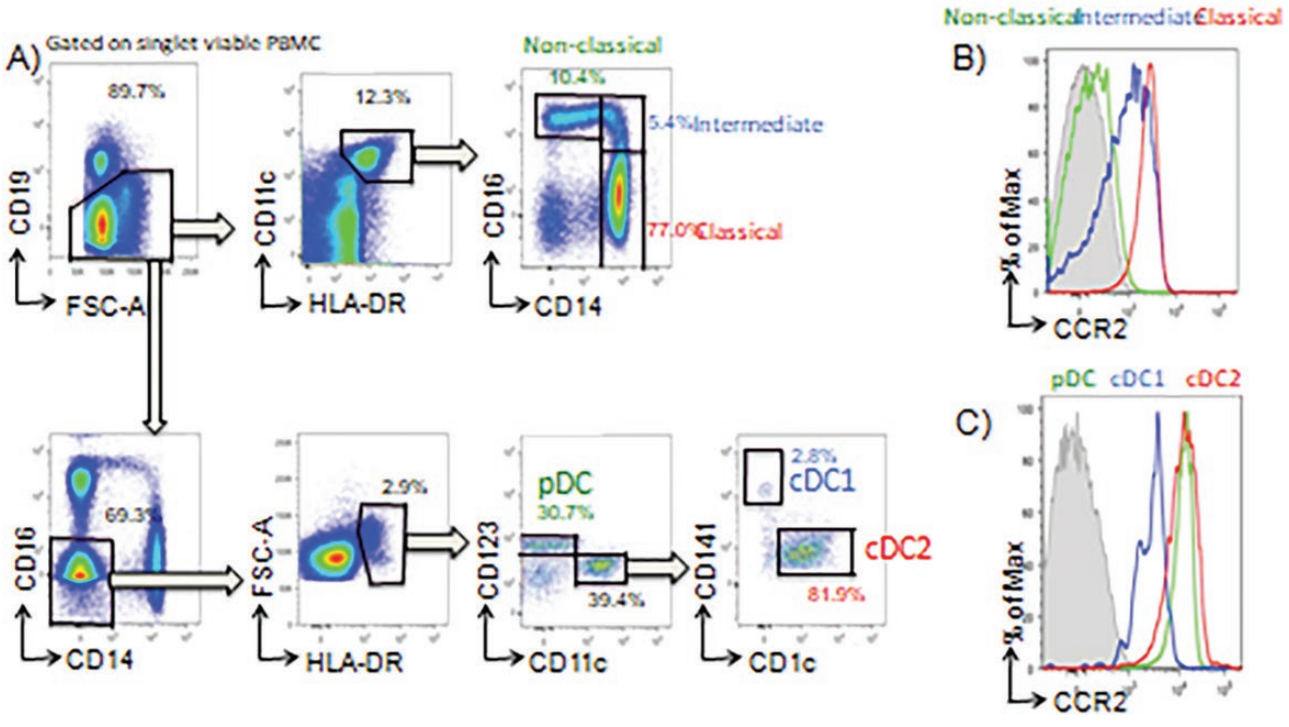

FIGURE 1. Cell subset identification. A, Monocyte and DC subsets were identified within singlet viable PBMCs. Monocytes were identified within the $CD19^-$ fraction as $HLA\text{-}DR^+CD11c^+$ and further divided into classical ($CD14^+CD16^-$), intermediate ($CD14^+CD16^+$), and nonclassical ($CD14^-CD16^+$) monocytes. In addition, DC were also identified within the $CD19^-$ fraction as $CD14^-CD16^-HLA\text{-}DR^+$. Plasmacytoid DC (pDC) were identified as $CD123^+$ and myeoloid or classical DC (cDC) were defined as $CD11c^+$. The latter were further divided into type 1 (cDC1) and type 2 (cDC2) cDC based on the expression of CD141 and CD1c, respectively. Surface expression or the different markers was further determined on each monocyte and DC subsets. B, Monocyte subset expression for each marker was determined as in the case of CCR2. Results were referred to the FMO, denoted as the shaded histogram. The MFI for each marker on each subset was divided by the specific FMO of each subset to obtain an IR of expression. C, The IR of expression for each marker (CCR2 in this case) on each DC subset was determined as in panel B, dividing the FMI of the marker by the specific FMO.

Indeed, similar results were obtained if the analysis was performed over the DC (Fig. 2B) or monocyte subsets (Fig. 2C), hence confirming the presence of homing- and activation-based differences between circulating DC and monocyte subsets from healthy CP and patients with different IBD types and mucosal status.

For deeper insight into these differences, we next aimed to identify the specific markers responsible for such differentiation to assess their relative contribution to IBD pathogenesis and their potential utility as biomarkers. Hence, IRs for each marker on each cell subset within each IBD condition were referred to their respective levels in the healthy CP population. However, when the markers were studied as single factors within each subset, no differences were found in IBD patients for any studied marker in pDC, cDC2, or intermediate monocytes (data not shown). As shown in Table 2, regarding the cDC1 subset, CCR3 expression was strongly associated with aCD. However, CCRL1 from both cDC1 and nonclassical monocytes seemed to display the opposite effect on patients with aUC. In a similar manner, CCR5 expression on cDC1 and β7 expression on classical monocytes were inversely associated with the presence of qUC. Moreover, CCR9 expression on classical monocytes was also inversely associated with qCD. When all DC and monocyte subsets were considered at the same time, the same homing markers, excluding β7, were still associated with IBD in the same manner (Table 3).

Having therefore identified the markers that better discriminated between the 5 studied populations (CCRL1, CCR3, and CCR5 on cDC1; CCRL1 on nonclassical monocytes; and CCR9 and β7 on classical monocytes; Table 2), we assessed whether a canonical analysis of these markers on these cell subsets might have the capacity to discriminate between healthy CP and patients with different IBD types (Fig. 3). However, the power of discrimination was lost because the studied groups overlapped. Hence, our results suggest that the differential profile found among the different DC and monocyte (Fig. 2) subsets between healthy CP and IBD patients did not result from the effect of the markers differentially expressed among the studied groups but rather because of several minor cumulative effects reflected in the differential profile of the groups.

DISCUSSION

We have described how human circulating DC and monocyte subsets display a differential profile between healthy CP and IBD patients. Indeed, such differential phenotypes of

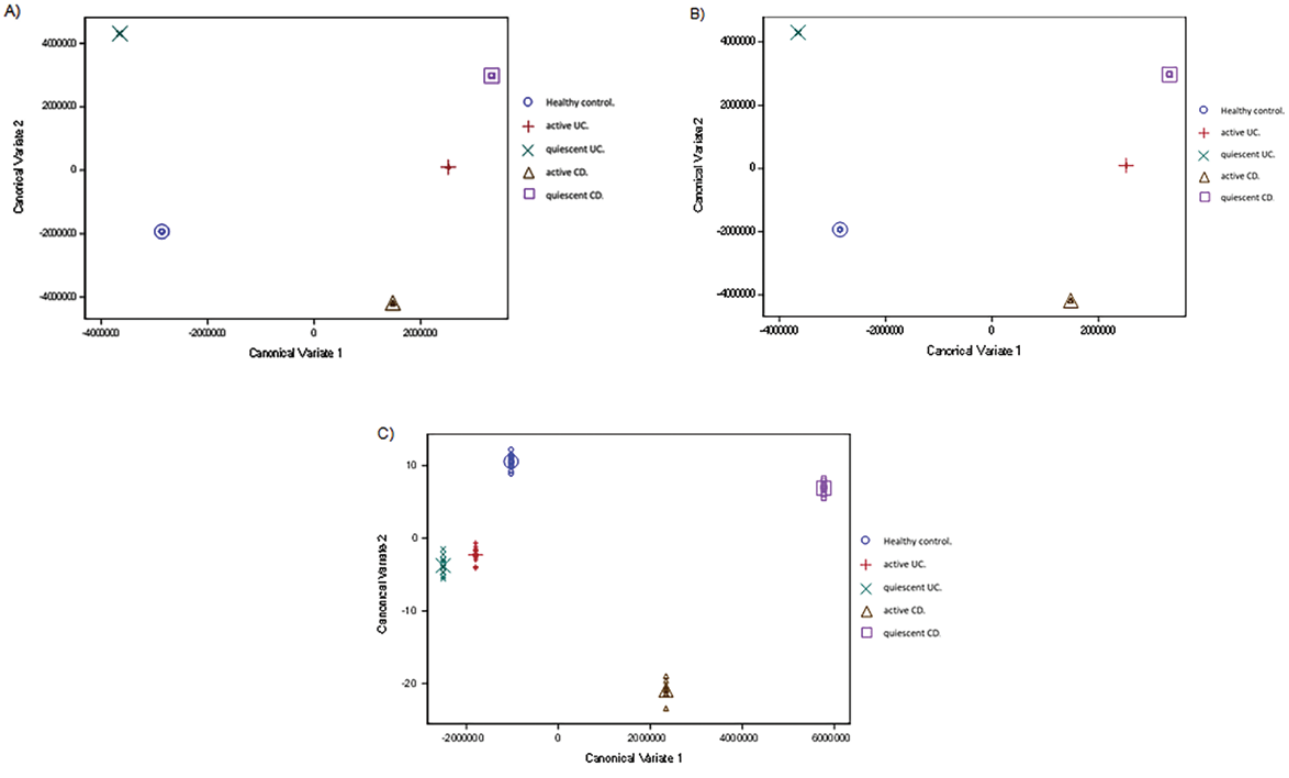

FIGURE 2. Discriminant canonical analysis performed using the 18 studied markers as discriminatory variables in the pool of monocyte and DC subsets (A), DC subsets (B), and monocyte subsets (C). Healthy CP, patients with active UC, patients with quiescent UC, patients with active CD, and patients with quiescent CD are denoted with big symbols for each group representing the centroid (mean vector and SD of each independent variable) and small symbols representing each individual patient. Note that all individuals in panels (A) and (B) fall within the centroid of their respective group.

TABLE 3. Association Between the Expression of Surface Markers in All DC and Monocyte Subsets in Patients With IBD

	Active UC		Quiescent UC		Active CD		Quiescent CD	
Marker	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***
CCR9	—	—	—	—	—	—	0.66 (0.47-0.91)	0.01
CCR3	—	—	—	—	2.29 (1.11-4.74)	0.02	—	—
CCR5	—	—	0.10 (0.01-0.83)	0.03	—	—	—	—
CCRL1	0.24 (0.08-0.66)	0.006	—	—	—	—	—	—

OR indicates odds ratio.

human circulating DC and monocytes allowed us to discriminate between both IBD type (CD or UC) and mucosal status (inflamed or noninflamed). These results therefore open the possibility of finding novel noninvasive biomarker approaches that could predict, by simply monitoring the profile of circulating DC and/or monocyte subsets, the mucosal status of IBD patients, hence avoiding the need for an invasive colonoscopy.

This study shows an association between migration and activation markers expressed on circulating DC and monocyte subsets with different IBD types, confirming a link between immune system cells and this pathology.[9] In addition, and although several studies have suggested that the role of homing markers influences IBD pathogenesis,[11, 12, 28–31] we suggest their use as possible biomarkers in IBD.[32] However, although the study of circulating DC and monocyte subsets can discriminate between IBD type (CD or UC) and mucosal status (inflamed or noninflamed) in the healthy CP population, this differentiation does not seem to be because of the presence of major factors. On the contrary, such differentiation seems to be derived from the accumulation of several minor effects, which, although interesting to analyze for deeper insight into the mechanisms governing IBD pathogenesis under different scenarios may not be

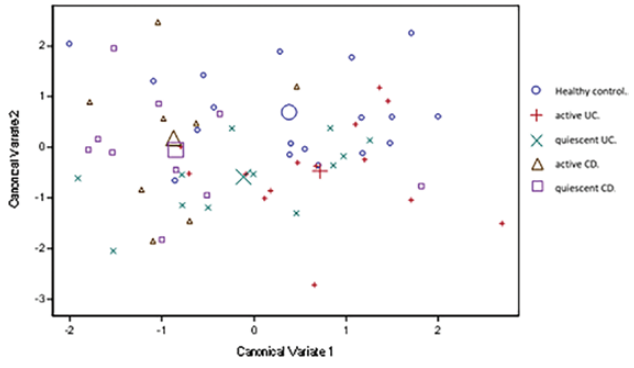

FIGURE 3. Discriminant canonical analysis based on the expression of CCRL1, CCR3, and CCR5 from type 1 conventional DC, CCRL1 expression from nonclassical monocytes, and CCR9 and β7 expression from classical monocytes as discriminatory variables.
Healthy CP, patients with active UC, patients with quiescent UC, patients with active CD, and patients with quiescent CD are denoted with big symbols for each group representing the centroid (mean vector and SD of each independent variable) and small symbols representing each individual patient.

of interest as noninvasive biomarkers. Nevertheless, after we began work on this study, other research reported that cDC and monocytes can be further divided into more subsets that those originally studied in this article.[33] Hence, we cannot discard the notion that if DC and monocyte subset characterization was performed on such a basis, then more robust results may have been obtained in our study.

Among all the studied markers on all the subsets, the expression of CCR3 on cDC1 was strongly associated with aCD. Given that CCR3 knockout mice show lower traffic of eosinophils to the intestinal mucosa than their control littermates,[10, 34] we cannot disregard a similar mechanism governing cDC1 migration toward human intestinal mucosa, which could be more prominent in CD than in UC.

Conversely, the expression of β7 on classical monocytes, CCR5 expression on cDC1, CCRL1 expression on cDC1, and nonclassical monocyte and CCR9 expression on classical monocytes seemed to elicit a protective function over IBD. Although it has been suggested that an increased expression of CCR5 on intermediate monocytes from patients with qCD with implications of monocyte migration, activation status, and production of colony stimulating factor-1 and interleukin-10,[31] in our cohort CCR5 expression was associated with the cDC1 subset but in the context of qUC. Indeed, an anti-inflammatory property has been suggested for this chemokine by down-modulating T cell–dependent immune response in mice.[35] Research has shown that CCRL1, also called ACKR4, is an atypical chemokine receptor that controls leukocyte migration,[36] transporting such homeostatic chemokines as CCL19, CCL21, CCL25, and CXCL13.[37] Some murine models of chronic intestinal inflammation have reported that a reduced expression of CCL21 affects DC migration and that a loss of CCL21 caused ileitis in mice.[38]

The chemokine receptor CCR9 mediates leukocyte migration toward the small bowel in a CCL25-dependent manner. Hence, patients with CD affecting the ileum but not the colon display higher levels of this marker on circulating cDC,[28] suggesting a role for this marker for ileal CD but not colonic IBD (UC or colonic CD). In fact, Peake et al[28] showed that CCR9 and CLA could stratify colonic- and ileal-affected patients, whereas these markers were not differentially expressed between the IBD groups in our study. That may be because we were not able to separate our patients into colonic or ileal disease categories because we did not have a large enough sample size of patients with ileal disease patients to perform any analysis. Another option to consider is that we studied patients with endoscopically inflamed or noninflamed disease (determined by the simplified endoscopic activity score for CD), whereas in the Peake et al study[28] CD patient classification into active or quiescent was based on the CD Activity Index in the absence of endoscopic assessment.[28] Last, we cannot discard that our discrepancies could result from environmental, geographical, or ethnic differences. Nevertheless, murine models of intestinal inflammation have shown beneficial effects of CCR9 expression in ileitis (resembling human CD).[39]

Finally, integrin β7 mediates, in conjunction with the α4 integrin, leukocyte migration toward the intestinal mucosa in a MadCAM-1 dependent fashion, being the target of the currently approved vedolizumab treatment. Hence, our results suggest that monocytes and DC infiltrated the intestinal mucosa in a β7-independent manner, the reason why its expression on monocytes was not associated with IBD as they infiltrated the mucosa via CCR2.[9] Altogether, this result highlights the relevance of these migration markers in the pathogenesis of IBD; their profile in circulating DC and monocyte subsets may also reveal differences between IBD types and mucosal status. Indeed, this conclusion is in agreement with the recent study from Rubin et al[40] that evaluated leukocyte subset functions and a gut-homing profile using CyTOF approaches, revealing the importance of gut trafficking molecules and pointing toward blood-based immune signatures that can differentiate clinical subsets of IBD.

We are nevertheless aware of the limitations of our study. One of them is that no tissue samples during colonoscopy were obtained from the patients, so we were not able to establish a correlation between our results in blood and intestinal tissue. Another limitation is that we did not assess chemokine levels but rather the expression of their receptors on circulating DC and monocyte subsets. A final limitatiom was the scarce statistical power to determine stronger associations between markers and IBD risk than those we found; this was partially because our study is a single-center study that only included a small cohort of Spanish patients as a proof of concept, lacking an independent sample on which to replicate our study. Our small cohort size was because we were aiming for environmental homogeneity of the patients recruited in a well-defined geographical region, hence abrogating the effects of environmental

factors on the immune system. Indeed, we consider that the large number of studied markers on several different immune cell subsets provides a strength to our study that can provide a background on which future similar studies can be based. Indeed, and given that we can discriminate among the different IBD types based on the profiling of circulating DC and monocyte subsets (Fig. 2), future studies can expand this approach to identify the most reliable markers that allow clinicians to discriminate among the different IBD types in the absence of a colonoscopy.

CONCLUSIONS

In summary, we describe how the profiling of human circulating DC and monocyte subsets discriminates between different IBD types. However, this discrimination was not because of the effect of any major marker(s) on some defined subset(s). On the contrary, the discrimination among the different study groups likely resulted from several minor cumulative effects reflected in the differential profile of the studied groups. Indeed, those minor effects allowed us to discriminate not just between CD and UC, but also between patients with endoscopically active or quiescent disease, suggesting the presence of different immunopathogenic pathways (in comparison to healthy CP) operating in these cohorts. Future studies will therefore have deeper insight into these mechanisms, hence aiming not only to decipher differences between the different IBD types but also to explore such variability as novel noninvasive biomarkers to aid in IBD diagnosis or monitoring.

SUPPLEMENTARY DATA

Supplementary data are available at *Inflammatory Bowel Diseases* online.

REFERENCES

1. Loftus EV Jr. Clinical epidemiology of inflammatory bowel disease: incidence, prevalence, and environmental influences. *Gastroenterology.* 2004;126:1504–1517.
2. M'Koma AE. Inflammatory bowel disease: an expanding global health problem. *Clin Med Insights Gastroenterol.* 2013;6:33–47.
3. Ng SC, Shi HY, Hamidi N, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *Lancet.* 2018;390:2769–2778.
4. Chaparro M, Barreiro-de Acosta M, Benítez JM, et al. EpidemIBD: rationale and design of a large-scale epidemiological study of inflammatory bowel disease in Spain. *Therap Adv Gastroenterol.* 2019;12:1–11.
5. Jones GR, Lyons M, Plevris N, et al. IBD prevalence in Lothian, Scotland, derived by capture-recapture methodology. *Gut.* 2019;68:1953–1960.
6. Kaplan GG, Ng SC. Understanding and preventing the global increase of inflammatory bowel disease. *Gastroenterology.* 2017;152:313–321.e2.
7. Charo IF, Ransohoff RM. The many roles of chemokines and chemokine receptors in inflammation. *N Engl J Med.* 2006;354:610–621.
8. Hart AL, Ng SC, Mann E, et al. Homing of immune cells: role in homeostasis and intestinal inflammation. *Inflamm Bowel Dis.* 2010;16:1969–1977.
9. Bernardo D, Marin AC, Fernández-Tomé S, et al. Human intestinal pro-inflammatory CD11chighCCR2+CX3CR1+ macrophages, but not their tolerogenic CD11c-CCR2-CX3CR1- counterparts, are expanded in inflammatory bowel disease. *Mucosal Immunol.* 2018;11:1114–1126.
10. White GE, Iqbal AJ, Greaves DR. CC chemokine receptors and chronic inflammation–therapeutic opportunities and pharmacological challenges. *Pharmacol Rev.* 2013;65:47–89.
11. Wendt E, Keshav S. CCR9 antagonism: potential in the treatment of inflammatory bowel disease. *Clin Exp Gastroenterol.* 2015;8:119–130.
12. Manousou P, Kolios G, Valatas V, et al. Increased expression of chemokine receptor CCR3 and its ligands in ulcerative colitis: the role of colonic epithelial cells in in vitro studies. *Clin Exp Immunol.* 2010;162:337–347.
13. Zundler S, Neurath MF. Novel insights into the mechanisms of gut homing and antiadhesion therapies in inflammatory bowel diseases. *Inflamm Bowel Dis.* 2017;23:617–627.
14. Coskun M, Vermeire S, Nielsen OH. Novel targeted therapies for inflammatory bowel disease. *Trends Pharmacol Sci.* 2017;38:127–142.
15. Paramsothy S, Rosenstein AK, Mehandru S, et al. The current state of the art for biological therapies and new small molecules in inflammatory bowel disease. *Mucosal Immunol.* 2018;11:1558–1570.
16. Hart AL, Al-Hassi HO, Rigby RJ, et al. Characteristics of intestinal dendritic cells in inflammatory bowel diseases. *Gastroenterology.* 2005;129:50–65.
17. Al-Hassi HO, Mann ER, Sanchez B, et al. Altered human gut dendritic cell properties in ulcerative colitis are reversed by *Lactobacillus plantarum* extracellular encrypted peptide STp. *Mol Nutr Food Res.* 2014;58:1132–1143.
18. Matsuno H, Kayama H, Nishimura J, et al. CD103+ dendritic cell function is altered in the colons of patients with ulcerative colitis. *Inflamm Bowel Dis.* 2017;23:1524–1534.
19. Sanders TJ, McCarthy NE, Giles EM, et al. Increased production of retinoic acid by intestinal macrophages contributes to their inflammatory phenotype in patients with Crohn's disease. *Gastroenterology.* 2014;146:1278–1288.e1.
20. Baumgart DC, Metzke D, Guckelberger O, et al. Aberrant plasmacytoid dendritic cell distribution and function in patients with Crohn's disease and ulcerative colitis. *Clin Exp Immunol.* 2011;166:46–54.
21. Silva MA, López CB, Riverin F, et al. Characterization and distribution of colonic dendritic cells in Crohn's disease. *Inflamm Bowel Dis.* 2004;10:504–512.
22. Dige A, Magnusson MK, Öhman L, et al. Reduced numbers of mucosal DR(int) macrophages and increased numbers of CD103(+) dendritic cells during anti-TNF-α treatment in patients with Crohn's disease. *Scand J Gastroenterol.* 2016;51:692–699.
23. Guilliams M, Ginhoux F, Jakubzick C, et al. Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. *Nat Rev Immunol.* 2014;14:571–578.
24. Guilliams M, van de Laar L. A Hitchhiker's guide to myeloid cell subsets: practical implementation of a novel mononuclear phagocyte classification system. *Front Immunol.* 2015;6:406.
25. Mann ER, Bernardo D, Ng SC, et al. Human gut dendritic cells drive aberrant gut-specific t-cell responses in ulcerative colitis, characterized by increased IL-4 production and loss of IL-22 and IFNγ. *Inflamm Bowel Dis.* 2014;20:2299–2307.
26. Baumgart DC, Metzke D, Schmitz J, et al. Patients with active inflammatory bowel disease lack immature peripheral blood plasmacytoid and myeloid dendritic cells. *Gut.* 2005;54:228–236.
27. Baumgart DC, Thomas S, Przesdzing I, et al. Exaggerated inflammatory response of primary human myeloid dendritic cells to lipopolysaccharide in patients with inflammatory bowel disease. *Clin Exp Immunol.* 2009;157:423–436.
28. Peake ST, Bernardo D, Knight SC, et al. Homing marker expression on circulating dendritic cells correlates with different phenotypes of Crohn's disease. *J Crohns Colitis.* 2013;7:594–596.
29. Takedatsu H, Mitsuyama K, Matsumoto S, et al. Interleukin-5 participates in the pathogenesis of ileitis in SAMP1/Yit mice. *Eur J Immunol.* 2004;34:1561–1569.
30. Maerten P, Geboes K, De Hertogh G, et al. Functional expression of 4-1BB (CD137) in the inflammatory tissue in Crohn's disease. *Clin Immunol.* 2004;112:239–246.
31. Nieto JC, Zamora C, Cantó E, et al. CSF-1 regulates the function of monocytes in Crohn's disease patients in remission. *Sci Rep.* 2017;7:92.
32. Miles B, Abdel-Ghaffar KA, Gamal AY, et al. Blood dendritic cells: "canary in the coal mine" to predict chronic inflammatory disease? *Front Microbiol.* 2014;5:6.
33. Villani A, Satija R, Reynolds G. Single-cell RNA-Seq reveals new types of human blood dendritic cells, monocytes, and progenitors. *Science.* 2017;356:eaah4573.
34. Humbles AA, Lu B, Friend DS, et al. The murine CCR3 receptor regulates both the role of eosinophils and mast cells in allergen-induced airway inflammation and hyperresponsiveness. *Proc Natl Acad Sci U S A.* 2002;99:1479–1484.
35. Zhou Y, Kurihara T, Ryseck RP, et al. Impaired macrophage function and enhanced T cell-dependent immune response in mice lacking CCR5, the mouse homologue of the major HIV-1 coreceptor. *J Immunol.* 1998;160:4018–4025.
36. Thomson CA, van de Pavert SA, Stakenborg M, et al. Expression of the atypical chemokine receptor ACKR4 identifies a novel population of intestinal submucosal fibroblasts that preferentially expresses endothelial cell regulators. *J Immunol.* 2018;201:215–229.
37. Parsi B, Esmaeili A, Hashemi M, et al. Transient expression of recombinant ACKR4 (CCRL1) gene, an atypical chemokine receptor in human embryonic kidney (HEK 293) cells. *Mol Biol Rep.* 2016;43:583–589.
38. Mikulski Z, Johnson R, Shaked I, et al. SAMP1/YitFc mice develop ileitis via loss of CCL21 and defects in dendritic cell migration. *Gastroenterology.* 2015;148:783–793.e5.
39. Wurbel MA, McIntire MG, Dwyer P, et al. CCL25/CCR9 interactions regulate large intestinal inflammation in a murine model of acute colitis. *PLoS One.* 2011;6:e16442.
40. Rubin SJS, Bai L, Haileselassie Y, et al. Mass cytometry reveals systemic and local immune signatures that distinguish inflammatory bowel diseases. *Nat Commun.* 2019;10:2686.