Original Article

Innate Immunity

Innate Immunity
2017, Vol. 23(6) 557–565

DOI: 10.1177/1753425917721965
journals.sagepub.com/home/ini
SAGE

The influence of vitamin D on M1 and M2 macrophages in patients with Crohn's disease

Serge Dionne, Carl-Frederic Duchatelier and Ernest G Seidman

Abstract

Defective bacterial clearance by macrophages plays an important role in Crohn's disease (CD). Phenotypes and functions of inflammatory M1 and anti-inflammatory M2 have not been studied in CD. Vitamin D supplementation reduces the severity of CD by unclear mechanisms. We studied macrophage characteristics in CD and controls and the effects of 1,25 vitamin D (1,25D). PBMC were isolated from CD patients and controls. M1 and M2 were generated by culturing of monocytes with GM-CSF and M-CSF, respectively. CD M1 and M2 showed normal phagocytosis and chemotaxis to CCL2 and fMLP. LPS-induced production of TNF-α, IL-12p40 and IL-10 was comparable between groups. Phagocytosis was unaltered with 1,25D; migration only increased marginally. M1 produced more IL-12p40 and TNF-α; IL-10 was greater in M2. 1,25D markedly decreased IL-12p40 by M1 and M2. 1,25D decreased TNF-α in CD M1; IL-10 levels were unaffected. M2 express F13A1, PTGS2, CD163, CXCL10, CD14 and MMP2, whereas TGF-β, CCL1 and CYP27B1 expression was higher in M1. Marker expression was similar between CD and controls. M1 and M2 markers were not differentially modulated by 1,25D. CD macrophages are not functionally or phenotypically different vs. controls. 1,25D markedly decreased pro-inflammatory M1 cytokines but did not modulate polarization to anti-inflammatory M2 phenotype.

Keywords

Vitamin D, macrophages, Crohn's disease, cytokines, innate immunity

Date received: 16 January 2017; revised: 18 May 2017; accepted: 23 June 2017

Introduction

Inflammatory bowel disease (IBD) is a chronic, relapsing inflammatory disorder of the gastrointestinal tract resulting from a disruption of immune tolerance to the intestinal microflora, leading to mucosal damage in genetically predisposed individuals.[1,2] Crohn's disease (CD) and ulcerative colitis (UC) are the two major types of IBD.[3] The composition of the intestinal microflora in IBD differs from healthy individuals, primarily owing to a lower incidence of dominant commensals (e.g. Firmicutes, *Bacteroides*, *Bifidobacterium*).

IBD is estimated to affect over 3.6 million persons in North America and Europe, and the incidence is increasing in Asia and Africa.[4,5] Genetic studies have identified >200 susceptibility loci for IBD, most of which are shared between CD and UC.[6–8]

The etiology of CD is poorly understood, with increasing evidence suggesting that it results from impaired innate immunity and mucosal barrier dysfunction leading to bacterial persistence and mucosal inflammation.[1,2,9] Defective autophagy associated with delayed elimination of intracellular bacteria has been implicated in the chronic intestinal inflammation in CD.[10–12] The *NOD2* gene is involved in the innate immune response and is highly associated with CD risk. Defects in NOD2 function can affect microbial sensing,[13] Paneth cell function and antimicrobial peptide (AMP) production,[14] as well as intracellular bacterial killing.[12]

Centre of Excellence in IBD, Research Institute of the McGill University Health Centre, Division of Gastroenterology, Montreal, Quebec, Canada

Corresponding author:
Ernest G Seidman, Canada Research Chair in Immune Mediated Gastrointestinal Disorders, Bruce Kaufman Endowed Chair in IBD at McGill Centre of Excellence in IBD, Research Institute of McGill University Health Center, 1650 Cedar Avenue, C10.145, Montreal, Quebec, Canada, H3G 1A4.
Email: ernest.seidman@mcgill.ca

The intestine encounters more Ags than any other organ and is thus home to the largest compartment of the immune system. Mononuclear phagocytes, including both dendritic cells and macrophages (Mϕ), play a central role in discriminating detrimental from harmless Ags. They are major effector cells of innate immunity, responsible for initiating and sustaining protective immune responses mounted towards pathogenic organisms, whilst also ensuring that local and systemic tolerance is generated in response to innocuous Ags. A breakdown of Mϕ tolerance against dietary proteins and the resident commensal microbiota is thought to lead to chronic inflammatory disorders such as CD.[15] Mϕ play an important role in maintaining tissue homeostasis, by clearing apoptotic or senescent cells, and by repairing and remodeling tissue during wound healing.[16]

M1/M2 describes the two major and opposing activities of Mϕ. M1 activity inhibits cell proliferation and causes tissue damage, whereas M2 activity promotes cell proliferation and tissue repair.[15] The molecules primarily responsible for these 'fight' (NO) or 'fix' (ornithine) activities both arise from arginine, via enzymatic pathways (iNOS and arginase) that down-regulate each other.[17] M1 and M2 Mϕ promote Th1 and Th2 responses, respectively. Products of Th1 and Th2 responses (e.g. IFN-γ, IL-4) also down-regulate M2 and M1 activity, respectively. It is hypothesized that imbalanced M1 or M2 are involved in the pathogenesis of IBD.[17]

Multiple factors, including cytokines and microbial products, affect Mϕ polarization.[18] Classically activated M1 develop in response to pro-inflammatory factors such as IFN-γ, TNF-α, LPS and GM-CSF. In mice, they differentiate from Ly6C^{hi} monocytes and promote inflammation by producing high levels of TNF-α, IL-1, IL-2, IL-6 and IL-23. M2 are differentiated by various stimuli, including Th2 cytokines (e.g. IL-4 and IL-13), and anti-inflammatory cytokines such as IL-10 and TGF-β. They express scavenger receptors, mannose receptors and arginase; produce large amounts of IL-10; and promote resolution of inflammation and tissue remodeling.[18] Some phenotype markers used to define mouse M1 such as NOS2, as well as the M2 markers Arginase-1 and Ym1, are not differentially expressed by human Mϕ.[19–21] Studies of the transcription profile of differently polarized Mϕ should help to identify human markers.[22–24]

It has become increasingly apparent that the active form of vitamin D (vD), 1,25-dihydroxyvitamin D (1,25D), is not only important for calcium and bone homeostasis, but is also involved in regulation of the immune response.[25] 1,25D exerts a positive effect on innate immunity, enhancing antibacterial defense through stimulation of autophagy and the induction of specific cytokine responses and antibacterial proteins.[26,27] The immunomodulatory effects of vD also involve adaptive immunity via inhibition of Th1 and Th17 pathways.[28–30]

vD Deficiency is suggested to play a role in the pathogenesis of various immune-mediated diseases, including IBD.[29] vD Deficiency is common among patients with IBD, including those recently diagnosed.[31] An inverse association between disease activity and serum vD levels was observed in some studies.[31,32] Few studies have been carried out in patients with CD supplemented with vD, with inconsistent results.[33] This may, in part, be explained by the fact that doses of vD supplementation were too low to normalize serum concentrations. In a recent pilot study, the vast majority of patients with CD needed 5000 IU/d to obtain adequate serum concentrations.[34]

Recently, it was reported that inhibiting M1 polarization and increasing M2 improved experimental colitis.[35,36] Scant information is available on alterations of Mϕ populations in human IBD. The mechanisms governing their effect on colitis development are unclear. The aims of this study were to examine M1 and M2 functions in CD vs. Mϕ from controls, and determine the effect of 1,25D on polarized Mϕ.

Material and methods

Patients and isolation of monocytes

Patients were recruited at the Centre of Excellence in IBD at the McGill University Health Center, Montreal, QC, Canada. Healthy controls consisted of age- and sex-matched individuals. Informed consent was obtained as per the protocol approved by the Research Ethics Board of the McGill University Health Centre (study number 14-499BMD). This research study involved human subjects and was conducted in accordance with the World Medical Association's Declaration of Helsinki. Patients taking vD supplementation were excluded from study. Overall, 44 patients with CD and 33 controls were included.

PBMC were isolated from heparinized venous blood using density-gradient centrifugation with Ficoll-Paque PLUS (GE Healthcare, Baie d'Urfe, QC, Canada) and plated at a concentration of 3×10^6 cells/ml. Monocytes were isolated using CD14 microbeads, MS columns and the VarioMACS Separation System (Miltenyi Biotec, Auburn, CA, USA).

Differentiation of monocytes into macrophages and stimulation

Monocytes were isolated using CD14 microbeads and plated at a concentration of 5×10^5 cells/ml. Inflammatory M1-type Mϕ were generated by culturing monocytes in the presence of GM-CSF, whereas

M-CSF (800 U/ml; Peprotech, Rocky Hill, NJ, USA) was used to produce anti-inflammatory M2. After 6 d, M1 and M2 Mϕ were pre-incubated for 20 h with 12.5 nM 1,25D. Mϕ were then used or were activated with LPS for an additional 24 h. Supernatants were collected and stored at −80°C until cytokine levels were determined by ELISA (R&D Systems, Minneapolis, MN, USA).

Phagocytosis

Differentiated Mϕ were cultured with different concentrations of 1,25D. Phagocytosis was measured using the Phagocytosis Assay kit (Cayman Chemical Co., Ann Arbor, MI, USA). In brief, FITC-latex beads were added to macrophages and cultured at 37°C for 30 min and then analyzed by flow cytometry (FACSCalibur; BD Biosciences, San Jose, CA, USA). Alternatively, phagocytosis was determined by reading the fluorescence intensity on a plate reader (Infinite M200, Tecan Group, Männersdorf, Switzerland).

RNA extraction and real-time PCR

Total cellular RNA was isolated from differentiated Mϕ using RNeasy plus mini kit (Qiagen, Valencia, CA USA). cDNA was generated using Transcriptor First Strand cDNA (Roche Diagnostics, Laval, QC, Canada). Real-time RT-PCR was performed by a StepOnePLus RT-PCR (Life Technologies, Burlington, ON, Canada) using PerfeCTa SYBR green Fast Mix (Quanta Biosciences, Beverly, MA, USA). Primers were ordered from Integrated DNA Technologies (Coralville, IA, USA).

The relative expression of each gene was calculated by the ΔCt method, where ΔCt is the value obtained by subtracting the Ct value of GADPH mRNA from the ΔCt value of the target gene. The amount of target relative to the GADPH mRNA was expressed as $2^{-(\Delta Ct)}$.

Modified Boyden chamber chemotaxis

Macrophages were plated on culture inserts (8-μm pore; Corning, Corning, NY). fMLP 250 nM or CCL2 25 ng/ml was added to the lower chamber of a 24-well plate. The plate was then incubated (4 h, 37°C). The assay was terminated by detaching the filter and wiping to remove non-migrated cells from the filter top. Migrated cells were then fixed with 1% formalin and stained with hematoxylin. Migrated cells were then counted under a microscope.

Statistical analysis

Data were analyzed using GraphPad Prism, version 5.03 (GraphPad Software, La Jolla, CA, USA). All data are presented as median. The statistical significance of the differences induced by 1,25D was determined using the Wilcoxon matched-pairs signed rank test. The difference between groups was determined using the Mann–Whitney test. Difference between paired samples was assessed using the Wilcoxon signed-rank test. A P-value ≤ 0.05 was considered statistically significant.

Results

Patients recruited for the study

Overall, 44 patients with CD and 33 controls were included. Their demographic information and vD status are shown in Table 1. No differences in age or sex were found. The median 25OHD for the CD and controls was not different. vD deficiency or insufficiency was observed in 84% of CD and 75% of the control group (non-significant).

Table 1. Characteristics of the patients with CD and controls.

	CD	Controls
n	35	20
Females, *n* (%)	14 (40)	12 (60)
Mean (range) age at consent	32.8 (17–64)	51.8 (19–91)
Age at diagnosis (%)		
• A1 (<16)	31	40
• A2 (17–40)	69	35
• A3 (> 40)	0	25
CD treatment (%)		
• None, *n* (%)	24	0 (100)
• 5-ASA alone	9	–
• Thiopurine or methotrexate	9	–
• TNF-α inhibitor	55	–
• Oral corticosteroids	3	–
vD status (nmol/l)	% [mean (range)]	% [mean (range)]
• Deficient (≤50)	60 [42 (14–50)]	40 [45 (40–50)]
• Insufficient (51–75)	29 [65 (53–75)]	35 [66 (51–75)]
• Sufficient (>75)	11 [112 (101–122)]	25 [87 (80–96)]
CD location (%)		
• L1—terminal ileum	31	–
• L2—colon	23	–
• L3—ileocolonic	46	–
• L4—upper GI tract	0	–
CD clinical phenotype (%)		–
• B1—inflammatory	43	–
• B2—stricturing	37	–
• B3—penetrating	20	–
Perianal involvement (%)	11	–

Crohn's disease age, location and clinical phenotype as per Levine et al.[57]
5-ASA: 5-aminosalicylic acid; GI, gastrointestinal.

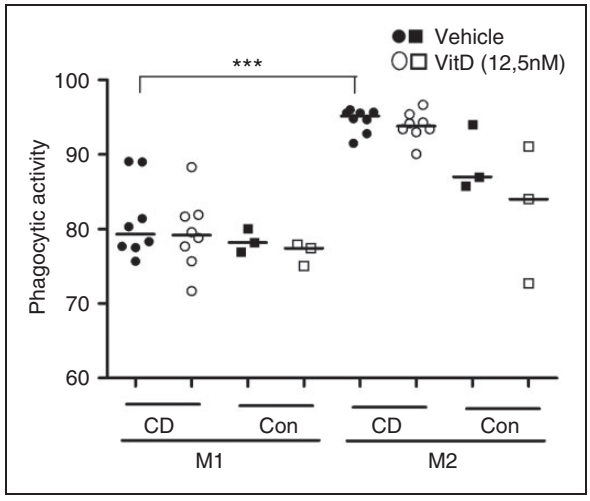

Figure 1. Effect of 1,25D on phagocytic activity of M1 and M2 macrophages generated from circulating monocytes in patients with CD ($n = 44$) and healthy controls (Con; $n = 33$). Macrophages were incubated with vehicle or vD (12.5 nM 1,25D) for 20 h. Phagocytosis of FITC-labeled latex beads was measured as described in the 'Materials and methods'. Data represent % of beads phagocytosed. $^{***}P < 0.001$.

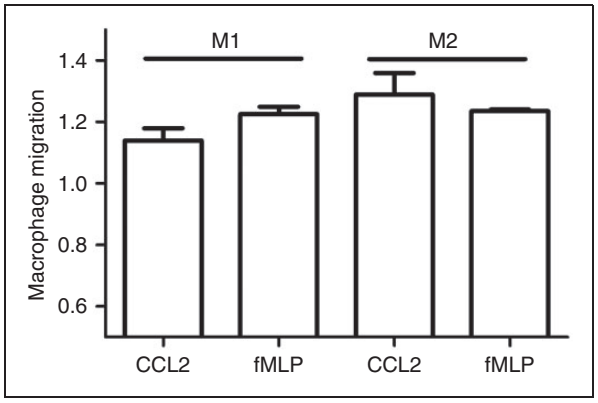

Figure 2. Macrophage chemotaxis in CD. M1 and M2 macrophages were generated from peripheral blood monocytes in 44 patients with CD as described in the 'Materials and methods'. Chemotaxis was measured by the modified Boyden method in response to CCL2 and fMLP, as described in the 'Materials and methods'. Data are presented as the number of migrated cells counted under a microscope in response to each chemokine relative to unstimulated conditions for the same patient.

Effect of 1,25D on phagocytosis and chemotaxis of macrophages

Phagocytic activity of M2 was greater than M1. However, phagocytic activity did not differ between Mϕ from patients with CD and controls. 1,25D had no effect on phagocytic activity by any macrophage population (Figure 1). The proportion of untreated and 1,25D-treated M2 that had phagocytosed latex beads was comparably high. Chemotaxis of M1 and M2 generated in CD are shown in Figure 2. M2 migrated in slightly higher numbers toward CCL2 compared with M1. No difference was seen in response to fMLP. 1,25D only marginally increased of both types of Mϕ toward fMLP and that of M2 towards CCL2 (data not shown).

1,25D inhibited inflammatory cytokines production by M1 and M2 macrophages

We next determined the effect of 1,25D on the production of anti- and pro-inflammatory mediators in Mϕ from patients with CD and healthy controls. We measured the production of TNF-α and IL-12/IL-23p40, two pro-inflammatory cytokines that play a central role in CD pathogenesis. M1 macrophages from patients with CD produced slightly more TNF-α than M2 macrophages ($P = 0.069$). LPS-induced TNF-α by CD and control Mϕ was comparable. 1,25D decreased TNF-α production by M1 from CD patients ($P < 0.005$) (Figure 3).

M1 produced much more IL-12/IL-23p40 than M2 macrophages ($P < 0.0005$) when stimulated with LPS (Figure 3). This is consistent with the known association between IL-12/IL-23p40 and M1 macrophages. M2 from patients with CD released less IL-12/IL-23p40 than those from controls ($P < 0.005$). Pre-incubation with 1,25D greatly decreased IL-12/IL-23p40 production by M1 macrophages from CD and controls ($P < 0.005$ and $P < 0.05$, respectively). IL-12/IL-23p40 released by M2 macrophages was also decreased that by 1,25D ($P < 0.005$).

IL-10 is an anti-inflammatory cytokine whose production is associated with M2 Mϕ.

As expected, IL-10 was produced in higher amounts by M2 than by M1 ($P < 0.005$). Mϕ from patients with CD and controls produced similar amounts of IL-10. Pre-incubation with 1,25D had no effect on IL-10 levels.

Effect of 1,25D on macrophage polarization markers

We next examined if 1,25D alters markers associated with M1 and M2. We found that CD80 and CCR7 cannot discriminate human M1 and M2, at least at the mRNA level. Using a recently published array,[37,38] we validated a set of genes that was preferentially expressed by M1 or M2 Mϕ. In CD, *F13A1*, *CD163*, *PTGS2* and *CD14* were preferentially expressed by M2 (Figure 4). M1 were associated with higher levels of *CYP27B1* and *CCL1* mRNA. Other genes, such as *MMP2*, *CXCL10*, *FN1* and *TGFB*, were also found to be expressed with some specificity to one of the Mϕ populations (Figure 4a). As cells were not always in sufficient numbers to generate both M1 and M2 Mϕ from the same patient, we analyzed the expression of the markers relative to GADPH as a

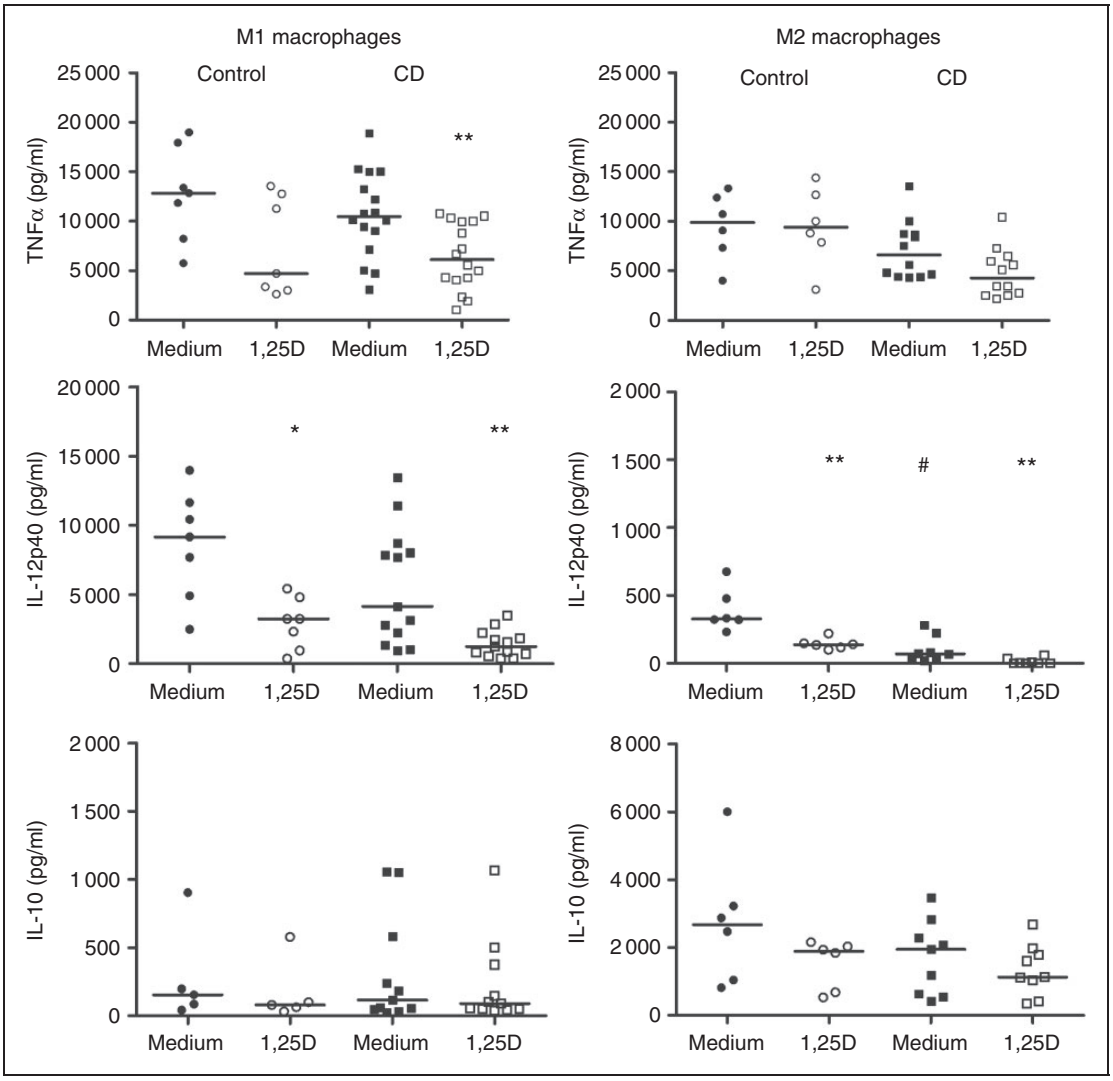

Figure 3. Effect of vD on cytokine production by M1 and M2 macrophages generated from peripheral blood monocytes in patients with CD ($n = 44$) and controls ($n = 33$). Polarized macrophages were incubated with vehicle or 12.5 nM 1,25D for 20 h. Cytokine levels were determined by ELISA after stimulation with 100 ng/ml LPS for 24 h. $^{*}P < 0.05$, $^{**}P < 0.005$, $^{***}P < 0.001$ vs. corresponding samples cultured without 1,25D; $^{\#}P < 0.05$ vs. control samples without 1,25D.

housekeeping gene. Although there was a loss in the power to discriminate M1 and M2 by relying on transcript abundance, several markers can still be used. Marker expression in controls and patients with CD was generally similar. PTGS2 expression tended to be higher in patients with CD than in controls, whereas M2 from controls seemed to express more CCL1 (Figure 4b).

We then evaluated the effect of 1,25D on Mϕ polarization markers in CD and controls. Only one of the M1 markers was modulated by 1,25D. CCL1 expression was increased twofold after incubation with 1,25D. However, this difference did not achieve statistical significance ($P = 0.06$; Figure 5), owing to the limited sample size available for analysis.

None of the M2 markers was decreased by 1,25D. Expression of CD14 and MMP2 by M2 was enhanced following 1,25D treatment, but the differences were not significantly different, likely owing to the small number of samples analyzed.

Discussion

Macrophages are one of the most abundant leukocytes in the intestinal mucosa, where they play an essential role in maintaining homeostasis.[15] However, they are also implicated in the pathogenesis of disorders such as IBD, offering potential targets for novel therapies. Depending on the signals received, Mϕ can be pro- or anti-inflammatory, immunogenic or tolerogenic, and can repair or destroy tissue. Mϕ are classified as pro-inflammatory M1 or anti-inflammatory M2.[17,18] The role of M1 and M2 in IBD has not been extensively investigated. M1 contribute to experimental dextran

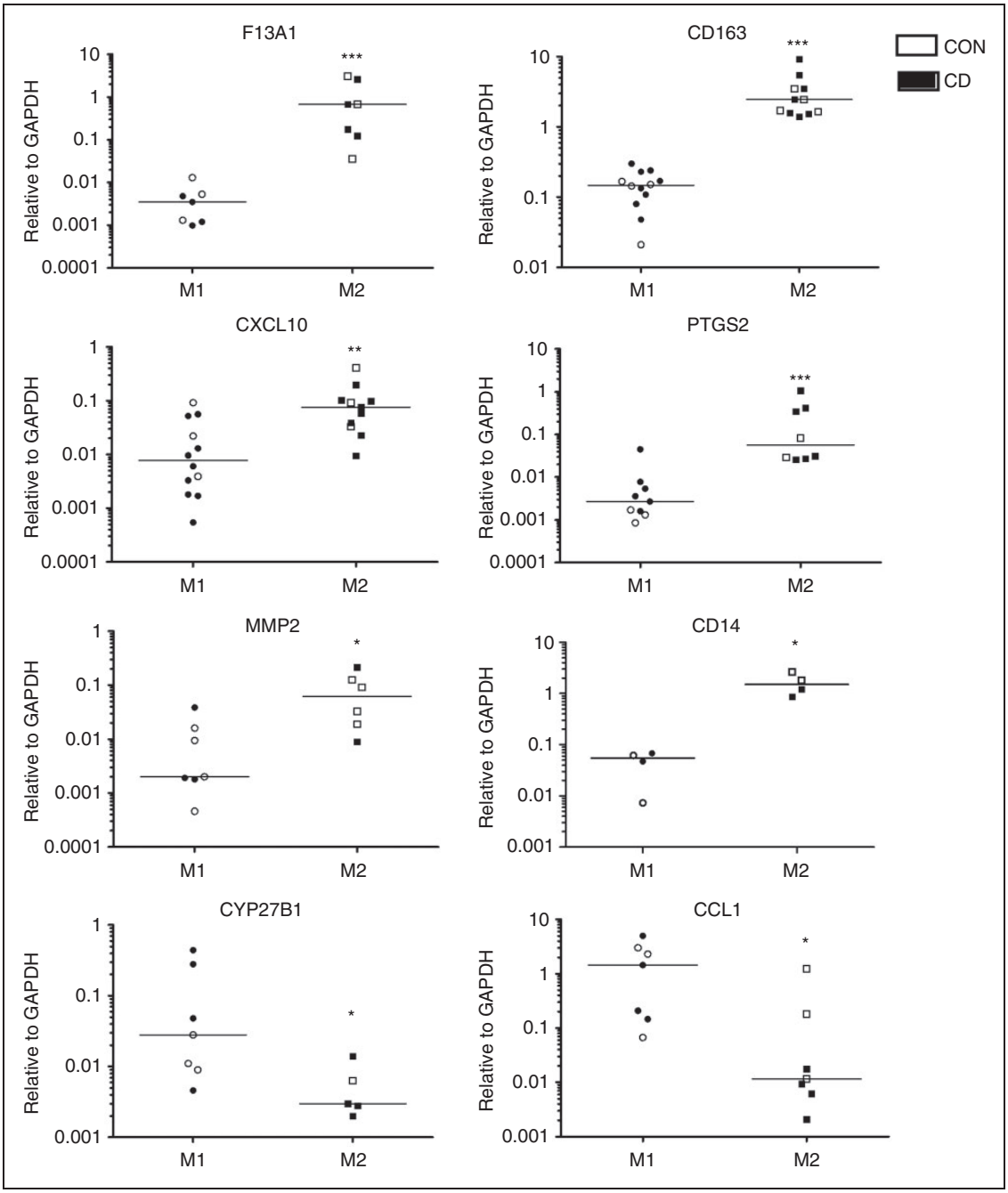

Figure 4. Verification of M1 and M2 markers for macrophages generated from peripheral blood monocytes in patients with CD ($n = 44$) and controls (Con; $n = 33$). (a) M2/M1 marker ratio in CD. (b) Marker transcript abundance relative to GADPH in CD and controls. *$P < 0.05$, **$P < 0.005$, ***$P < 0.001$.

sulfate sodium (DSS) colitis, whereas M2 macrophages attenuate colitis severity.[36] Inhibiting M1 polarization was reported to ameliorate colitis.[36,39] Using iNOS and TNF-α as M1 markers and CD163 and stabilin-1 as M2 markers, Lissner et al.[40] provided evidence that Mϕ subtypes in the intestinal lamina propria are shifted towards pro-inflammatory M1 in CD. Using a co-culture model, M1 reduced transepithelial resistance as a marker for epithelial barrier integrity.[40] The mechanisms for paracellular leakage included intracellular re-localization of tight junction proteins and epithelial cell apoptosis. Using specific cytokine blockade, it was found that M1 exerted their deleterious effect mainly through TNF-α.[40] Using double-labelling immunohistochemistry *in situ*, Barros et al.[41] also reported that M1 were higher in Th1- associated diseases such as CD. In this study, we examined if M1 and M2 Mϕ from patients with CD differed in phenotype and function from those of control patients, and whether vD can modulate their function *in vitro*.

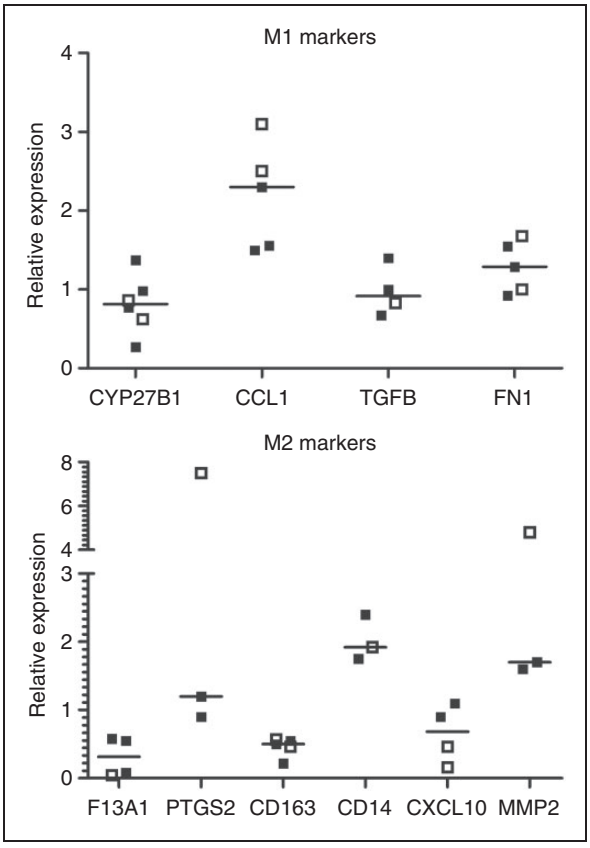

Figure 5. Effect of 1,25D on M1 and M2 polarization marker expression in macrophages from patients with CD (■) and controls (□). Macrophages were derived from peripheral blood monocytes in patients deficient in vD. Changes in M1 markers induced by 12.5 nM 1,25D for 20 h are shown in the upper panel. Changes in M2 markers are similarly illustrated in the lower panel.

Our data reveal that Mϕ from control and patients with CD display comparably high phagocytic ability. Chemotactic response of CD and control Mϕ was also similar. Our results are in line with a recent study that showed that monocyte-derived Mϕ isolated from patients with CD and healthy controls display a similar ability to kill phagocytosed bacteria and produce equivalent cytokine levels in response to *Escherichia coli*.[42] Similarly, phenotypic and functional studies on peripheral blood monocytes from patients with CD in clinical remission were not found to be impaired compared with healthy controls.[43] These results, taken together, suggest that CD, rather than associated with an underlying immunodeficiency, may be related to defective innate immune mechanisms in the inflamed intestinal mucosa rather than in peripheral blood.

Tissue-resident M2 Mϕ contribute to mucosal tolerance by secreting IL-10.[44] The lamina propria of the inflamed intestine in patients with IBD, especially CD, is massively infiltrated by monocytes and proinflammatory M1 Mϕ.[40] Infiltrating intestinal macrophages are distinct in phenotype and function from their resident counterparts. They strongly express CD14, TREM-1, an amplifier of pro-inflammatory responses, and the human myeloid IgA Fc receptor CD89.[44] In addition, they exhibit activated NF-κB and secrete pro-inflammatory cytokines such as TNF-α. These Mϕ are thought to contribute to IBD pathogenesis by disrupting the epithelial barrier.[40] However, Smith et al.[45] reported that in CD Mϕ, an abnormal proportion of cytokines are routed to lysosomes and degraded rather than being released through the normal secretory pathway, leading to impaired bacterial clearance. We did not observe significant differences in cytokine secretion between Mϕ from patients with CD and controls. TNF-α and IL-10 production by Mϕ from patients with CD was comparable with that of controls. LPS elicited a lesser amount of IL-12/IL-23p40 in CD M2 than controls. IL-12/IL-23p40 production by M1 had large inter-individual variations in both groups.

Studies on phenotypic characterization of M1 and M2 Mϕ in human diseases are limited. We observed that M1 were associated with higher levels of CYP27B1 and CCL1 mRNA levels. This is consistent with a previous study in which global gene expression profiles of Mϕ derived from human monocytes by GM-CSF or M-CSF were compared with their mouse counterparts.[22]

Once formed by CYP27B1, 1,25D carries out its diverse biological functions in the intestine, bone and kidney by signaling through the vD receptor. 1,25D is involved in many biological processes, such as apoptosis, cell proliferation and immune function.[46,47] As a modulator of innate and adaptive immunity, vD can affect carcinogenesis via changes in systemic inflammation, by down-regulating the TLR signals, cytokines, adipokines and chemokines responsible for Mϕ infiltration of adipose tissue.[48] vD modulates the composition of the gut microbiome and its deficiency provokes impaired epithelial integrity, as well as increased inflammation.[49–51] 1,25D stimulates innate immunity by inducing NOD2 expression and by stimulating genes encoding AMPs, such as defensins and cathelicidin.[52,53]

Low plasma levels of 25(OH)D are associated with an increased risk of CD, as well as colorectal cancer.[54] In a recent publication,[55] we reported that vD-deficient mice displayed more severe DSS colitis than vD-supplemented mice, with lower survival rates. Increased histological inflammation score and increased IL-6 were also observed in the mucosa of vD-deficient mice. Moreover, vD supplementation decreased the number of inflammation-associated colorectal tumors in mice, independent of the *NOD2* gene.[55]

CCL1 is a chemokine that attracts Th2 and T regulatory cells.[56] F13A1, CD163, PTGS2 and CD14 were preferentially expressed by M2 Mϕ. F13A1 encodes the

coagulation factor XIII A subunit that was previously associated with M2 Mϕ,[22] whereas the Mϕ scavenger receptor CD163 is a classic M2 marker. Inflammatory cytokines such as IL12p40 and TNF-α have been associated with M1, whereas production of IL-10 has been linked with M2. We investigated the potential of vD to modulate Mϕ function in patients with CD who were not taking supplements and who were mostly deficient (Table 1). The resulting data show that for both control and CD Mϕ, 1,25D decreased inflammatory cytokines while maintaining IL-10 production. Previous reports showed that 1,25D often stimulates IL-10 production, thereby suppressing inflammation. We recently reported that 1,25D pre-treatment decreased the production of TLR2-, TLR4- and TLR7/8-induced cytokines.[52] IL-12/IL-23p40 levels were reduced by 45–56%, whereas those of IL-23 were reduced by 64–70%. IL-10 was less affected, being decreased by up to 25%. These data are in keeping with the effects of 1,25D viewed herein. The different cell types studied, with dissimilar culture conditions and stimuli used can explain the inconsistent IL-10 response to vD *in vitro*.

Treatment with 1,25D did not change expression of M1 markers except for CCL1, which increased slightly. Expression of the two major M2 markers was not significantly altered by 1,25D for either group. There was a modest increase in PTGS2 and MMP2 following 1,25D incubation. CD14, a known vD receptor target, was upregulated. Several confounding factors may account for the lack of polarization of M1 to M2 in response to 1,25D. First and foremost is the limited number of patients with cells that were available after completing the other experiments. Other explanations include the process used to generate Mϕ, the culture conditions and concentration of 1,25D employed.

In summary, 1,25D treatment did not significantly affect Mϕ functions such as phagocytosis and chemotaxis, but exerted an inhibitory effect on pro-inflammatory cytokine production. Certain limitations exist in interpreting the results generated in this research. The cells studied were cultured and treated with agents to generate Mϕ. These manipulations may yield cells that do not necessarily reflect the real physiological or pathological status *in vivo*. Moreover, it would be of interest to study intestinal rather than peripheral Mϕ. However, the cell isolation process may alter Mϕ function. Nevertheless, we believe the data presented here will serve as a starting point for future studies into Mϕ biology in CD, ideally focusing on intestinal-derived cells.

Acknowledgements

Ernest Seidman is a Tier 1 Canada Research Chair in immune-mediated gastrointestinal disorders and holds the Bruce Kaufman Chair in IBD at McGill University.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This research was funded by a grant from the Dairy Farmers of Canada.

References

1. Abraham C and Medzhitov R. Interactions between the host innate immune system and microbes in inflammatory bowel disease. *Gastroenterology* 2011; 140: 1729–1737.
2. Imhann F, Vila AV, Bonder MJ, et al. Interplay of host genetics and gut microbiota underlying the onset and clinical presentation of inflammatory bowel disease. *Gut*. Epub ahead of print 8 October 2016. DOI: 10.1136/gutjnl-2016-312135.
3. Fava F and Danese S. Intestinal microbiota in inflammatory bowel disease: friend of foe? *World J Gastroenterol* 2011; 17: 557–566.
4. Molodecky NA, Soon S, Rabi DM, et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. *Gastroenterology* 2012; 142: 46–54.
5. M'Koma AE. Inflammatory bowel disease: an expanding global health problem. *Clin Med Insights Gastroenterol* 2013; 6: 33–47.
6. McGovern DP, Kugathasan S and Cho JH. Genetics of inflammatory bowel diseases. *Gastroenterology* 2015; 149: 1163–1176.
7. Liu JZ, van Sommeren S, Huang H, et al. Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. *Nat Genet* 2015; 47: 979–986.
8. Huang C, Haritunians T, Okou DT, et al. Characterization of genetic loci that affect susceptibility to inflammatory bowel diseases in African Americans. *Gastroenterology* 2015; 149: 1575–1586.
9. Corridoni D, Arseneau KO and Cominelli F. Functional defects in NOD2 signaling in experimental and human Crohn disease. *Gut Microbes* 2014; 5: 340–344.
10. Becker C, Neurath MF and Wirtz S. The intestinal microbiota in inflammatory bowel disease. *ILAR J* 2015; 56: 192–204.
11. Kuballa P, Huett A, Rioux JD, et al. Impaired autophagy of an intracellular pathogen induced by a Crohn's disease associated ATG16L1 variant. *PLOS ONE* 2008; 3: e3391.
12. Lapaquette P, Bringer MA and Darfeuille-Michaud A. Defects in autophagy favour adherent-invasive Escherichia coli persistence within macrophages leading to increased pro-inflammatory response. *Cell Microbiol* 2012; 14: 791–807.
13. Jiang W, Wang X, Zeng B, et al. Recognition of gut microbiota by NOD2 is essential for the homeostasis of intestinal intraepithelial lymphocytes. *J Exp Med* 2013; 210: 2465–2476.
14. Bevins CL, Stange EF and Wehkamp J. Decreased Paneth cell defensin expression in ileal Crohn's disease is independent of inflammation, but linked to the NOD2 1007fs genotype. *Gut* 2009; 58: 882–883.
15. Bain CC and Mowat AM. The monocyte-macrophage axis in the intestine. *Cellular Immunol* 2014; 291: 41–48.
16. Italiani P and Boraschi D. New insights into tissue macrophages: from their origin to the development of memory. *Immune Netw* 2015; 15: 167–176.
17. Mowat AM and Bain CC. Mucosal macrophages in intestinal homeostasis and inflammation. *J Innate Immun* 2011; 3: 550–564.
18. Mantovani A, Sica A and Locati M. Macrophage polarization comes of age. *Immunity* 2005; 23: 344–346.

19. Martinez FO, Gordon S, Locati M, et al. Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. *J Immunol* 2006; 177: 7303–7311.
20. Murray PJ and Wynn TA. Obstacles and opportunities for understanding macrophage polarization. *J Leukoc Biol* 2011; 89: 557–563.
21. Raes G, Van den Bergh R, De Baetselier P, et al. Arginase-1 and Ym1 are markers for murine, but not human, alternatively activated myeloid cells. *J Immunol* 2005; 174: 6561.
22. Lacey DC, Achuthan A, Fleetwood AJ, et al. Defining GM-CSF- and macrophage-CSF-dependent macrophage responses by in vitro models. *J Immunol* 2012; 188: 5752–5765.
23. Beyer M, Mallmann MR, Xue J, et al. High-resolution transcriptome of human macrophages. *PLOS ONE* 2012; 7: e45466.
24. Mills CD, Lenz LL and Ley K. Macrophages at the fork in the road to health or disease. *Front Immunol* 2015; 6: 59.
25. Hewison M. An update on vitamin D and human immunity. *Clin Endocrinol* 2012; 76: 315–325.
26. Chun RF, Liu PT, Modlin RL, et al. Impact of vitamin D on immune function: lessons learned from genome-wide analysis. *Front Physiol* 2014; 5: 151.
27. Verway M, Bouttier M, Wang TT, et al. Vitamin D induces interleukin-1β expression: paracrine macrophage epithelial signaling controls M. tuberculosis infection. *PLOS Pathog* 2013; 9: e1003407.
28. Peelen E, Knippenberg S, Muris AH, et al. Effects of vitamin D on the peripheral adaptive immune system: a review. *Autoimmun Rev* 2011; 10: 733–743.
29. Wöbke TK, Sorg BL and Steinhilber D. Vitamin D in inflammatory diseases. *Front Physiol* 2014; 5: 244.
30. Hayes CE, Hubler SL, Moore JR, et al. Vitamin D actions on CD4(+) T cells in autoimmune disease. *Front Immunol* 2015; 6: 100.
31. Ardesia M, Ferlazzo G and Fries W. Vitamin D and inflammatory bowel disease. *Biomed Res Int* 2015; 2015: 470805.
32. Hlavaty T, Krajcovicova A, Koller T, et al. Higher vitamin D serum concentration increases health related quality of life in patients with inflammatory bowel diseases. *World J Gastroenterol* 2014; 20: 15787–15796.
33. Mouli VP and Ananthakrishnan AN. Review article: vitamin D and inflammatory bowel diseases. *Aliment Pharmacol Ther* 2014; 39: 125–136.
34. Yang L, Weaver V, Smith JP, et al. Therapeutic effect of vitamin D supplementation in a pilot study of Crohn's patients. *Clin Transl Gastroenterol* 2013; 4: e33.
35. Arranz A, Doxaki C, Vergadi E, et al. Akt1 and Akt2 protein kinases differentially contribute to macrophage polarization. *Proc Natl Acad Sci U S A* 2012; 109: 9517–9522.
36. Chang HH, Miaw SC, Tseng W, et al. PTPN22 modulates macrophage polarization and susceptibility to dextran sulfate sodium-induced colitis. *J Immunol* 2013; 191: 2134–2143.
37. Xue J, Schmidt SV, et al. Transcriptome-based network analysis reveals a spectrum model of human macrophage activation. *Immunity* 2014; 40: 274–288.
38. Sudan B, Wacker MA, Wilson ME, et al. A systematic approach to identify markers of distinctly activated human macrophages. *Front Immunol* 2015; 6: 253.
39. Jang SE, Hyam SR, Han MJ, et al. *Lactobacillus brevis* G-101 ameliorates colitis in mice by inhibiting NF-κB, MAPK and AKT pathways and by polarizing M1 macrophages to M2-like macrophages. *J Appl Microbiol* 2013; 115: 888–896.
40. Lissner D, Schumann M, Batra A, et al. Monocyte and M1 macrophage-induced barrier defect contributes to chronic intestinal inflammation in IBD. *Inflamm Bowel Dis* 2015; 21: 1297–1305.
41. Barros MH, Hauck F, Dreyer JH, et al. Macrophage polarisation: an immunohistochemical approach for identifying M1 and M2 macrophages. *PLOS ONE* 2013; 8: e8090.
42. Flanagan PK, Chiewchengchol D, Wright HL, et al. Killing of *Escherichia coli* by Crohn's disease monocyte-derived macrophages and its enhancement by hydroxychloroquine and vitamin D. *Inflamm Bowel Dis* 2015; 21: 1499–1510.
43. Schwarzmaier D, Foell D, Weinhage T, et al. Peripheral monocyte functions and activation in patients with quiescent Crohn's disease. *PLOS ONE* 2013; 26: 8: e62761.
44. Kühl AA, Erben U, Kredel LI and Siegmund B. Diversity of intestinal macrophages in inflammatory bowel diseases. *Front Immunol* 2015; 6: 613.
45. Smith AM, Rahman FZ, Hayee B, et al. Disordered macrophage cytokine secretion underlies impaired acute inflammation and bacterial clearance in Crohn's disease. *J Exp Med* 2009; 206: 1883–1897.
46. Barragan M, Good M and Kolls JK. Regulation of dendritic cell function by vitamin D. *Nutrients* 2015; 7: 8127–8151.
47. Diaz L, Diaz-Munoz M, Garcia-Gaytan AC, et al. Mechanistic effects of calcitriol in cancer biology. *Nutrients* 2015; 7: 5020–5050.
48. Raman M, Milestone AN, Walters JR, et al. Vitamin D and gastrointestinal diseases: inflammatory bowel disease and colorectal cancer. *Therap Adv Gastroenterol* 2011; 4: 49–62.
49. Ooi JH, Li Y, Rogers CJ, et al. Vitamin D regulates the gut microbiome and protects mice from dextran sodium sulfate-induced colitis. *J Nutr* 2013; 143: 1679–1686.
50. Wang TT, Dabbas B, Laperriere D, et al. Direct and indirect induction by 1,25-dihydroxyvitamin D3 of the NOD2/CARD15-defensin beta2 innate immune pathway defective in Crohn disease. *J Biol Chem* 2010; 285: 2227–2231.
51. Zhao H, Zhang H, Wu H, et al. Protective role of 1,25(OH)2 vitamin D3 in the mucosal injury and epithelial barrier disruption in DSS-induced acute colitis in mice. *BMC Gastroenterol* 2012; 12: 57.
52. Dimitrov V and White JH. Species-specific regulation of innate immunity by vitamin D signaling. *J Steroid Biochem Mol Biol* 2016; 164: 246–253.
53. Dionne S, Calderon MR, White JH, et al. Differential effect of vitamin D on NOD2- and TLR-induced cytokines in Crohn's disease. *Mucosal Immunol* 2014; 7: 1405–1415.
54. Ananthakrishnan AN, Cheng SC, Cai T, Cagan A, et al. Association between reduced plasma 25-hydroxy vitamin D and increased risk of cancer in patients with inflammatory bowel diseases. *Clin Gastroenterol Hepatol* 2014; 12: 821–827.
55. Elimrani I, Koenekoop J, Dionne S, et al. Vitamin D reduces colitis-and inflammation-associated colorectal cancer in mice independent of NOD2. *Nutr Cancer* 2017; 69: 276–288.
56. Sironi M, Martinez FO, D'Ambrosio D, et al. Differential regulation of kine production by Fcgamma receptor engagement in human monocytes: association of CCL1 with a distinct form of M2 monocyte activation (M2b, Type 2). *J Leukoc Biol* 2006; 80: 342–349.
57. Levine A, Griffiths A, Markowitz J, et al. Pediatric modification of the Montreal classification for inflammatory bowel disease: the Paris classification. *Inflamm Bowel Dis* 2011; 17: 1314–1321.