Immunosuppressive Effects via Human Intestinal Dendritic Cells of Probiotic Bacteria and Steroids in the Treatment of Acute Ulcerative Colitis

Siew C. Ng, PhD,* Sophie Plamondon, MD,* Michael A. Kamm, MD,*,† Ailsa L. Hart, PhD,* Hafid Omar Al-Hassi, PhD,* Thomas Guenther, PhD,‡ Andrew J. Stagg, PhD,* and Stella C. Knight, PhD*

Background: In ulcerative colitis (UC) gut bacteria drive inflammation. Bacterial recognition and T-cell responses are shaped by intestinal dendritic cells (DCs); therapeutic effects of probiotic bacteria may relate to modulation of intestinal DC. The probiotic mixture, VSL#3, increases interleukin (IL)-10 and downregulates IL-12p40 production by DC in vitro. We evaluated in vivo effects of oral VSL#3 and steroids on colonic DC in patients with acute UC.

Methods: Rectal biopsies were obtained from patients with active UC before and after treatment with VSL#3, corticosteroids, or placebo, and from healthy controls. Myeloid colonic DC were studied from freshly isolated lamina propria cells using multicolor flow cytometry. Surface expression of activation markers, CD40, CD86, pattern recognition receptors, Toll-like receptor (TLR)-2 and TLR-4 were assessed. Changed function was measured from ongoing intracellular IL-10, IL-12p40, IL-6, and IL-13 production.

Results: Acute UC colonic myeloid DC were producing more IL-10 and IL-12p40 than control DC (P = 0.01). In VSL#3-treated patients DC TLR-2 expression decreased (P < 0.05), IL-10 production increased and IL-12p40 production decreased (P < 0.005); 10/14 patients on VSL#3 showed a clinical response. Corticosteroids also resulted in increased IL-10 and reduced IL-12p40 production by DC. Conversely, in patients on placebo, TLR-2 expression and intensity of staining for IL-12p40 and IL-6 increased (all P < 0.05); 5/14 patients on placebo showed a clinical response (P = NS).

Conclusions: Despite small numbers of human colonic DC available, we showed that treatment of UC patients with probiotic VSL#3 and corticosteroids induced “favorable” intestinal DC function in vivo, increasing regulatory cytokines and lowering proinflammatory cytokines and TLR expression. These effects may contribute to therapeutic benefit.

Key Words: ulcerative colitis, dendritic cells, probiotic bacteria, VSL#3, steroids, intestinal inflammation

Ulcerative colitis (UC) results from an inappropriate mucosal immune response to indigenous microbiota and luminal antigens in genetically susceptible individuals. Intestinal microbiota is altered in patients with active UC compared with that of healthy individuals. Manipulating gut flora using probiotics may correct and/or prevent proinflammatory signals in UC. VSL#3, a combination of 8 different probiotic bacterial strains, is effective in maintaining remission and preventing development of pouchitis. In an open-label study and a recent placebo-controlled trial, VSL#3 was effective in the treatment of patients with mild to moderately active UC, with a response rate of 50%–70%.

Dendritic cells (DCs) are key initiators of innate and adaptive immune responses present in small numbers in all tissues, including the gut. Responses of DC to microbial antigens are enabled partly through their rich supply of pattern recognition receptors such as Toll-like receptors (TLRs). Intestinal DCs are present within Peyer’s patches and distributed throughout the lamina propria. They produce veiled extensions into the gut lumen to sample antigens. Intestinal DC may be responsible for tolerance to commensal microorganisms, immune activation in response...
to pathogens, and inflammatory activity in inflammatory bowel diseases (IBDs) in humans. Most studies supporting a role for DC are based on animal models, but, over a number of years, we have developed methods for assessing phenotype and function of human DC from limited material available from human gut biopsies. Our work on human DCs provides evidence for their involvement in human gut inflammation and of the capacity of gut bacteria to regulate immune responses via human intestinal DC.

Altered DC phenotype and function in IBD suggests that they contribute to inflammatory processes. Colon DC from IBD patients are more activated and express increased levels of maturation markers and TLRs, more DCs also produce pathologically relevant cytokines. Intestinal DCs are, therefore, likely to be key initiators and perpetuators of inflammation in IBD.

DCs sample and respond to gut bacteria, and may be central factors in immunomodulation by these bacteria. An in vitro sonicated VSL#3 mixture, in particular its bifidobacterial components, induces interleukin (IL)-10 in DC from both blood and intestinal biopsies and inhibits generation of Th1 cells. It remains to be established whether in vivo administration of live probiotic bacteria, in the setting of resident microbiota and active mucosal inflammation, results in modulation of gut DC similar to that seen in vitro. We establish for the first time favorable in vivo effects of therapeutic intervention with bacterial modification (probiotics) and standard immunosuppression (corticosteroids) on phenotype and function of human colonic DC in acute UC, including correlations with therapeutic efficacy.

**PATIENTS AND METHODS**

**Patients**

Patients with mild to moderately active UC (n = 28) were recruited at a specialist hospital as part of a multicenter, double-blind, placebo-controlled trial evaluating efficacy of VSL#3. Healthy controls (n = 12; 6 female) with no history of bowel disease were also included. Results of the larger trial assessing the clinical effects of VSL#3 in acute UC will be reported separately. The main aim of the study reported here was to investigate the mechanism of action of VSL#3 on DC in a subgroup of patients in the clinical trial and to correlate these ex vivo laboratory findings with the clinical effects.

In the main clinical trial, for the purposes of study inclusion the diagnosis of UC was made using clinical, radiographic, endoscopic, and histologic criteria. Disease activity was assessed using the UC Disease Activity Index: UCDAI, with scores 0 (inactive disease) to 12 (maximum disease). Patients symptomatic for <4 weeks who had a UCDAI of 3–8 with a minimum sigmoidoscopic score of 2 on UCDAI were eligible. Tissue inflammation was confirmed by histological evaluation. Patients were on stable doses of medication. Exclusion criteria included the use of steroids within the last 4 weeks; use of antibiotics within the last 2 weeks; change in dose of 5-aminosalicylate acid (ASA) within the last 4 weeks; change in dose of rectal 5-ASA or steroids within 7 days prior to study entry, or the use of probiotic preparations during study. Rectal therapy was not permitted.

Healthy controls had macroscopically and histologically normal intestine (median age 51 years; range 22–60 years). The protocol was approved by the local ethics committee (VSL#3 Trial REC 2758) and informed consent was obtained from all patients.

**Study Medication**

VSL#3 (VSL Pharmaceuticals, Fort Lauderdale, FL) sachets contained 900 billion viable lyophilized bacteria consisting of 4 strains of lactobacillus (L. casei, L. Plantarum, L. acidophilus, L delbrueckii subsp. bulgaricus), 3 strains of Bifidobacterium (B. longum, B. breve, B. infantis), and 1 strain of Streptococcus salivarius subsp. thermophilus. The placebo was provided in the form of identical sachets containing maize starch. Each patient received 2 sachets twice/day of VSL#3 (3600 billion bacteria), or placebo, for 8 weeks. Patients treated with oral corticosteroids received prednisolone 40 mg daily, reduced by 5 mg each week over 8 weeks.

**Clinical Definition**

The UCDAI is the sum of scores from 4 criteria (stool frequency, rectal bleeding, sigmoidoscopic mucosal appearance, physician’s global assessment). Clinical remission was defined as a UCDAI of ≤2 points. Clinical response was defined as a decrease in UCDAI ≥3 points and a final score ≤3.

**Grading of Histologic Inflammation**

Severity of histologic inflammation in up to 2 rectal biopsies was reported by an expert pathologist (T.G.). Severity of inflammation was graded histologically as described by Gupta et al. Degree of inflammation was graded as: 0, inactive/quiescent/normal (no epithelial neutrophil infiltration); 1, mild (neutrophil infiltration of <50% of sampled crypts/cross-sections, no ulcers/erosions); 2, moderate (neutrophil infiltration of ≥50% of sampled crypts or cross-sections, no ulcers/erosions; or 3, severe (erosions/ulceration).

**Isolation of Lamina Propria Mononuclear Cells**

The method, detailed in our previous work, utilized up to 10 rectal biopsies per subject obtained at sigmoidoscopy/
colonoscopy. Biopsies were collected in ice-cold RPMI 1640 Dutch modification (Sigma-Aldrich, Dorset, UK) supplemented with 10% fetal calf serum, 25 μg/mL gentamicin, and 100 U/mL penicillin/streptomycin (complete medium). Tissue was incubated in calcium- and magnesium-free Hank’s balanced salt solution (HBSS) (Gibco BRL, Paisley, UK) containing 1 mmol/L dithiothreitol (Sigma-Aldrich) for 20 minutes in a T25 tissue culture flask. Epithelium was removed using 1 mmol/L EDTA in HBSS at 37°C with gentle agitation.

Tissue was digested for 90–150 minutes at 37°C using 25 mL of 1 mg/mL collagenase D (Roche Diagnostics, Lewes, UK), 20 μg/mL deoxyribonuclease I (Roche Diagnostics), and 2% fetal calf serum in RPMI 1640 Dutch modification (Sigma-Aldrich, Dorset, UK) supplemented with 10% fetal calf serum, 25 μg/mL gentamicin, and 100 U/mL penicillin/streptomycin (complete medium). Released lamina propria mononuclear cells (LPMCs) were washed in complete medium. Released lamina propria mononuclear cells (LPMCs) were labeled in calcium- and magnesium-free phosphate-buffered saline (PBS) containing 1 mmol/L EDTA and 0.02% sodium azide (fluorescence-activated cell sorter FACS buffer). A minimum of 50,000 LPMCs were used per antibody labeling. Labeling was performed on ice, cells were washed twice by centrifugation in FACS buffer (300g, 10 min, 4°C), and resuspended in 200 μL of paraformaldehyde (500 μL of a 1.0% weight/volume solution in 0.85% saline) and stored at 4°C until acquisition on the FACS within 24 hours.

Intracellular Cytokine Production by Lamina Propria DC

Paired cultures, 1 without monensin and the other with monensin to maintain cytokine within the Golgi apparatus of cells, were cultured for 4 hours at 37°C in a humidified atmosphere of 5% CO₂ in air. Cells were labeled for surface markers, fixed with leucoperm A, and permeabilized with leucoperm B. Anti-cytokine antibody was added for 30 minutes on ice. The cells were washed and fixed in 1% paraformaldehyde.

Multiplex Enzyme-linked Immunoabsorbent Assay (ELISA)

Supernatants were harvested from a whole biopsy cultured overnight in medium. Cytokine contents of supernatants were measured using Multiplex Fluorescent Bead Immunoassay (human Th1/Th2 10plex Kit II BMS716FF, Bender Medsystems, Austria) for quantitative detection by flow cytometer of 10 different cytokines: interferon gamma (IFN-γ), IL-12, IL-10, IL-8, IL-6, IL-4, IL-5, IL1-beta, tumor necrosis factor alpha (TNF-α), IL-12p70 in tissue culture supernatants from UC patients before and after 8 weeks treatment with VSL#3, steroids, or placebo. The 25-μL aliquots of supernatant were pipetted into a 96-well filter bottom microplate and incubated with 25 μL mixed antibody beads and 50 μL biotinylated conjugate mixture per well in the dark for 2 hours at room temperature on a plate shaker (500 rpm). After washing, beads were stained with streptavidin-PE (50) for 1 hour in the dark on a plate shaker. After further 2 washes with 100 μL assay buffer using vacuum manifold to aspirate, 200 μL of assay buffer was added to each well, the contents were mixed, and transferred into a FACS tube. The samples were stored at 4°C and acquired within 24 hours. The quantities of cytokine in the supernatant were determined against a standard curve for known concentrations of that cytokine.

Flow Cytometry

Data were acquired using a FACS Calibur flow cytometer (Becton Dickinson, Oxford, UK). Live cells were analyzed on HLA-DR versus lineage cocktail staining. Lineage cocktail comprised specific monoclonal antibodies for T cells (CD3), monocytes (CD14, CD16), B cells (CD19), and stem cells (CD34). Using multicolor analysis, CD11c+ populations within an HLA-DR+ lin⁻/dim gate were assessed for expression of cell surface markers.

The percentage of cells expressing a surface marker was measured from proportion of antibody stained cells falling beyond the distribution of staining with an isotype-matched control antibody. Level of staining for surface markers, expressed as an intensity ratio (IR) representing the ratio of median value of positive events in the test histogram to median value of staining with an isotype-matched control antibody was measured using Enhanced Normalized Subtraction (ENS) on WinList software (Verity Software House, Topshame, ME).

The percentage of cytokine-positive cells was determined by superenhanced Dmax (SED) normalized subtraction. Normalized cumulative histograms of staining of cells cultured without monensin (control) were subtracted from histograms of staining with monensin (test histogram). Both samples were treated in the same way to ensure minimal difference in nonspecific binding between test and
control samples. Using ENS from Winlist software, cytokine production was determined by the subtraction of staining in the sample with monensin from staining in sample without monensin. When more “positive events” in the control histogram were detected compared with test histogram, subtraction was reversed to measure loss of cytokine (negative events). The optimization of this technique to detect ongoing cytokine production in unstimulated DC has been described and this sensitive technique may complement both secretion and gene expression techniques.\textsuperscript{23} Level of staining (IR) for cytokine was determined using ENS. Absolute cells counts were obtained by simultaneous acquisition of Flow-Count fluorospheres (Coulter Immunotech, Hialeah, FL).

### Statistical Analysis

Stata (v. 9.2) by StataCorp (College Station, TX) software was used for statistical analyses. Pooled data were expressed as median values ± standard mean error. Two-tailed t-tests were employed to compare normally distributed data and Mann–Whitney Rank-Sum tests were used for non-normally distributed data. Pre- and posttreatment values were compared using the Wilcoxon matched-pair test. Association between variables was examined using Pearson correlation. For most data, values $P < 0.05$ were considered significant, whereas to allow for multiple testing for multiplex analysis, only a $P$-value $< 0.01$ was considered statistically significant.

### RESULTS

#### Clinical Outcome of Patients

Baseline characteristics of patients are shown in Table 1. There were no significant differences between the VSL#3 and placebo group for any demographics examined.

Fourteen patients were randomized to VSL#3 and 14 to placebo. Ten of 14 patients on VSL#3 responded to treatment compared with 5 of 14 on placebo ($P = 0.064$).

<table>
<thead>
<tr>
<th>TABLE 1. Baseline Characteristics of UC Patients</th>
<th>VSL#3</th>
<th>Placebo</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>4/10</td>
<td>7/7</td>
<td>0.44</td>
</tr>
<tr>
<td>Mean age, year (range)</td>
<td>45 (21–70)</td>
<td>41 (28–57)</td>
<td>0.54</td>
</tr>
<tr>
<td>Disease duration, years(range)</td>
<td>5 (1–16)</td>
<td>7 (1–27)</td>
<td>0.44</td>
</tr>
<tr>
<td>Disease extent</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distal</td>
<td>7</td>
<td>8</td>
<td>1.00</td>
</tr>
<tr>
<td>Extensive</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Mean UCDAI, range</td>
<td>7 (4–8)</td>
<td>6 (5–8)</td>
<td>0.32</td>
</tr>
<tr>
<td>Histological activity index</td>
<td>1.6</td>
<td>2.1</td>
<td>0.11</td>
</tr>
<tr>
<td>Concomitant medications (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mesalazine only</td>
<td>5 (36)</td>
<td>9 (64)</td>
<td>0.16</td>
</tr>
<tr>
<td>AZA/6-MP</td>
<td>2 (14)</td>
<td>3 (21)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>7 (50)</td>
<td>2 (14)</td>
<td></td>
</tr>
</tbody>
</table>

Mesalazine: Stable for at least 4 weeks.
AZA/6MP: Stable for at least 12 weeks.

A clinical response was defined as a decrease in UCDAI of at least 3 units. Ten of 14 patients treated with VSL#3 responded to treatment compared with 5 of 14 on placebo ($P = 0.064$).

FIGURE 1. Clinical response by treatment arm.
Fisher’s Exact Test). Two and 6 patients from the VSL#3 and placebo group, respectively, withdrew from the trial within 1 week due to lack of therapeutic efficacy or worsening disease and were treated with oral corticosteroids. In the VSL#3 group, intention to treat analysis, that is, including those who withdrew within the first week of treatment, demonstrated that remission (UCDAI/C20) was achieved in 7 (50%) patients, clinical response in a further 3 (21%) patients, no response in 3 (21%) patients, and 1 (7%) patient did not have the final sigmoidoscopy assessment. In patients who had placebo, remission was seen in 5 (36%), response in 0 (0%), and no response in 9 (64%). Figure 1 shows the patients’ clinical response by study arm.

Characteristics of Human Colonic DC in Acute UC

Identification of Intestinal DCs

Lamina propria DCs were identified from colonic tissue as HLA-DR+ lineage−/dim (lin = anti-CD3, CD14, CD16, CD19, CD34) populations. Within the DC gate, CD11c+ and CD11c− cells were present. (B) Labeling of gated DC subsets with surface markers was quantified using the region gating method. A region is drawn that excludes irrelevant isotype-matched control antibody (R1) and nonspecific staining and all events that exceed isotype control staining intensity were regarded as positively labeled cells (R2). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

![Image of DC gating and cytokine production](https://www.interscience.wiley.com)

Fisher’s Exact Test). Two and 6 patients from the VSL#3 and placebo group, respectively, withdrew from the trial within 1 week due to lack of therapeutic efficacy or worsening disease and were treated with oral corticosteroids. In the VSL#3 group, intention to treat analysis, that is, including those who withdrew within the first week of treatment, demonstrated that remission (UCDAI ≤2) was achieved in 7 (50%) patients, clinical response in a further 3 (21%) patients, no response in 3 (21%) patients, and 1 (7%) patient did not have the final sigmoidoscopy assessment. In patients who had placebo, remission was seen in 5 (36%), response in 0 (0%), and no response in 9 (64%). Figure 1 shows the patients’ clinical response by study arm.

Characteristics of Human Colonic DC in Acute UC

Identification of Intestinal DCs

Lamina propria DCs were identified from colonic tissue as HLA-DR+ Lin−/dim population. Within this gate, CD11c+ myeloid and CD11c− populations were present (Fig. 2A). Studies were performed on CD11c+ myeloid DC, the best-characterized and immunologically understood DC in human gut. The gating method to quantify labeling with surface markers is demonstrated (Fig. 2B).

More Colonic CD11c+ DC Produced IL-10 and IL-12p40 in UC than Control

Ongoing intracellular cytokine production by colonic CD11c+ DC was assessed without exogenous stimulation. In acute UC a significantly greater proportion of CD11c+ DC produced IL-10 and IL-12p40 than equivalent cells from control tissue, where there was no detectable IL-10 or IL-12p40 (Fig. 3A). Levels of cytokine staining (mean positive intensity ratios) of IL-10 and IL-12p40 by CD11c+ DC were also significantly greater in UC tissue than control tissue (Fig. 3B). No significant differences were observed for IL-6 or IL-13 production by DC between UC and control tissues. We measured IL-13, as this Th2
cytokine is associated with UC; nonclassical natural killer (NK) T cells in the lamina propria of UC produced IL-13.

Expression of costimulatory molecules and TLRs on colonic DC was not significantly different between UC patients and controls (UC versus controls: CD40: 72 ± 5% versus 77 ± 7%; CD86: 58 ± 5% versus 59 ± 6%; TLR-2: 14 ± 3% versus 25 ± 7%; TLR-4: 12 ± 2% versus 15 ± 5%).

Effects of Therapy on DC Phenotype and Functions

**VSL#3 Downregulated TLR-2 Expression on CD11c+ Colonic DC**

There was no significant difference between TLR-2 expression on colonic DC in the VSL#3 or placebo groups before treatment but TLR-2 expression was significantly reduced after VSL#3. In contrast, there were no significant differences.
differences in CD40, CD86, or TLR-4 expression after treatment (Fig. 4A). In patients on placebo, there were no significant changes in DC expression of CD40, CD86, TLR-2, and TLR-4 (Fig. 4B).

**VSL#3 Increased IL-10 and Inhibited IL-12p40 Production by Colonic DC**

Significantly increased IL-10 production and decreased IL-12p40 production by colonic DC were seen following VSL#3 treatment, but there were no significant changes in IL-6 or IL-13 production (Fig. 5A). Treatment with placebo was not associated with any significant changes in proportion of cytokine producing DC (Fig. 5B), but there was a significant increase in level of staining for IL-12p40 and IL-6 on CD11c+ DC (Fig. 5C).

Eight patients had a clinical response and 2 patients had no response to VSL#3. We assessed whether changes in DC cytokine production was associated with clinical outcome in patients treated with VSL#3. The 2 patients who had no clinical response after treatment with VSL#3 showed a less marked posttreatment reduction in IL-12p40 (mean reduction 16% in IL-12p40+ DC after 8 weeks treatment) than did DC from VSL#3 responders (mean reduction 94% in IL-12p40+ DC, \( n = 8 \)). In addition, VSL#3 responders demonstrated an increased proportion of IL-10+ DC (mean increase 83%), whereas in nonresponders there was a mean reduction of 14% in IL-10+ DC. Although these numbers are modest, these data suggest an association between change toward beneficial DC cytokine profile and clinical efficacy.

In VSL#3 responders (\( n = 8 \)), mean positive intensity ratio (PIR) of IL-10 increased from 1.7 to 2.4, whereas PIR IL-12 reduced from 2.4 to 0.7. In nonresponders, PIR of IL-10 and IL-12p40 were unchanged post-VSL#3. In contrast, in 5 patients on placebo who had a clinical response, mean PIR of IL-10 was unchanged, whereas PIR IL-12p40 increased from 1.2 to 1.8. In 4 patients who had placebo who did not respond, PIR of IL-10 reduced and IL-12p40 increased.

**Oral Corticosteroids Enhanced IL-10 and Inhibited IL-12p40 Production by Colonic DC**

There was a reduction in colonic CD11c+ DC production of IL-12p40 after treatment with corticosteroids (\( P \)
FIGURE 5. Cytokine production by colonic DC before and after treatment with VSL#3. (A) Paired samples of colonic DC from patients with acute UC, obtained before and after VSL#3 treatment, were stained for the presence of intracellular IL-10, IL-12p40, IL-6, and IL-13. The proportion (%) of cytokine-positive DC is shown. (B) Paired samples of colonic DC from patients with acute UC, obtained before and after placebo treatment, were stained for the presence of intracellular IL-10, IL-12p40, IL-6, and IL-13. The proportion (%) of cytokine positive DC is shown. Solid symbols represent patients with no clinical response and open symbols represent patients with a clinical response or remission. (C) Intensity of cytokine staining (PIR) in colonic DC before and after VSL#3 or placebo.

*P<0.05
= 0.012). Conversely, IL-10 production by CD11c+ DC increased after steroid therapy (P = 0.032) (Fig. 6). Median levels of IR for IL-12p40 decreased from 1.7 to 1.0, while median IR for IL-10 increased from 1.3 to 1.8 (P = 0.04). There were no significant changes in CD11c+ DC IL-6 or IL-13 production (not shown).

Correlation Between Initial UCDAI and Changes in DC

There was no significant correlation between patients’ UCDAI score (range 4–8, median 6) at baseline and baseline measurement of percentage positive cells for phenotypic markers or intracellular cytokines.

Tissue Histologic Activity Index Pre- and Posttreatment

Patient’s pre- and posttreatment tissue was scored for histological inflammatory activity (Fig. 7). In all treated patients there was no significant change in mean histologic activity index (VSL#3: 1.6 to 1.8; P = 0.41; placebo 1.9 to 1.2; P = 0.098; steroids 2.0 to 1.0; P = 0.17). The mean histology score after VSL#3 between responders and nonresponders were unchanged at 1.8 and 2.0, respectively. Histological changes are often slower to manifest than symptom and endoscopic or macroscopic improvement. In 9 of 10 patients, inflammatory infiltrate remained in tissue post-VSL#3. Similarly, in patients on steroids altered cytokine profile occurred irrespective of resolution of inflammatory cell infiltrate. In 5 patients who had steroids, an inflammatory cell infiltrate was still present on histology (score 1–3) but changes were detected in cytokine profiles.

Multiplex ELISA of Cytokines in Tissue Supernatant

In all UC patients, cytokines including IL-10, IL-8, IL-6, IL-4, IL-5, IL-1β, TNF-α, and IL-12p70 were detected in supernatants from tissue cultures. There was no significant difference in amounts of cytokines (in pg/ml/mg) in supernatants before and after VSL#3 or steroid treatment (Table 2). The unchanged inflammatory cell activities in supernatants measured by ELISA, together with a lack of histological change posttreatment, suggest that changes in DC cytokine production are unlikely to result from resolution of inflammatory cell infiltrates, but may reflect altered function of specific cell populations.

DISCUSSION

We have shown for the first time that in vivo treatment with VSL#3 of patients with acute UC induced IL-10 and downregulated IL-12p40 production in colonic DC; these findings are in line with previously published in vitro data that VSL#3 is a potent inducer of IL-10 by blood and lamina propria DC.14 Treatment with oral VSL#3 and corticosteroids, but not placebo, modulated human colonic DC toward a regulatory/antiinflammatory profile, suggesting that probiotic and steroid effects on DC are likely to be central to their therapeutic effects in UC.

The main objective of these studies relates to mechanistic actions of VSL#3 and corticosteroids on DC, which may underlie their known clinical effects. These mechanistic studies were performed in a subgroup of patients who were part of a larger clinical trial; results of the larger trial assessing the clinical effects of VSL#3 in acute UC will be presented separately at a future date.

DC represent only around 0.2% of viable cells from human gut biopsies and the numbers of DC are therefore limited; our development and refinement over many years of techniques to identify, purify, and study their functions were central to this project.13,25 DCs are likely to be pivotal in mediating the balance between effector and regulatory lymphocyte responses with different outcomes influenced by exposure of DC to microbial products. Immature DC from nonintestinal sites express low levels of CD40 but this activation marker is upregulated when DCs undergo maturation or activation as occurs in inflamed mucosa of patients with IBD.15,26 We specifically measured TLR-2 and TLR-4, as previous work has shown that colonic myeloid DC have enhanced expression of TLR-2 and TLR-4 in IBD, especially in inflamed mucosa of patients with Crohn’s disease (CD).15 However, in this study we observed no difference in expression of costimulatory molecules and TLRs between UC patients and controls, which may relate, in part, to specific characteristics of the patient population studied. For instance, all patients had mild to moderately active UC, and most patients were on some form of stable antiinflammatory drugs including mesalazine or azathioprine. Comparative data between patient population (i.e., mild versus severe disease; treatment-naïve patients versus patients on immunosuppressive therapy) will be important.

DCs respond differentially to different types of microbes TLR.27–29 Soluble factors such as cytokines or bacterial products can modulate TLR expression. In murine models of colitis, the antiinflammatory effect of probiotics may be mediated via TLR-9,30 and possibly via TLR-2 as shown here. Downregulation of TLR-2 by DC after VSL#3 may reflect reduced inflammation after treatment, although this effect was not seen with resolution of inflammation after placebo, or be secondary to downstream local immune modulation or recruitment of inactivated cells from blood. The signaling of VSL#3 via TLR-2 on DC may result in IL-10 production. TLR-2 knockout mice display defective production of IL-10.31 In addition, Candida albicans induces immunosuppression through TLR2-derived signals that mediate increased IL-10 production and survival of “T
reg" cells. It has also been shown that DC expression on TLR results in a noninflammatory response.

Acute UC was associated with increased production of both IL-10 and IL-12p40 by colonic myeloid DC, contrasting with the normal proportion of IL-12p40-producing DC we found in chronic UC. In acute mucosal inflammation, IL-10 and IL-12p40 may act synergistically, failing to suppress acute inflammatory response by IL-10 and inducing IL-12-mediated inflammation. Alternatively, an increase in both regulatory and proinflammatory cytokines may represent feedback inhibition of Th2 inflammation. Recent genome-wide association scanning has also demonstrated a susceptibility gene specific for UC related to IL-10. In our study there was lack of IL-13 production by DC; DC production of IL-13 may increase if measured in later stages of inflammation, or the cellular source of IL-13 may not be DC; in animal studies mucosal cytokines differ between induction and maintenance phases of intestinal inflammation. Synergism between production and effects of Th1 and Th2 cytokines has been described. In chronic 2,4,6-trinitrobenzene sulfuric acid colitis, fibrosis that occurs in the later stage of disease is dependent on development of an IL-13 response by NK T cells.

Patients with UC treated with oral VSL#3 had increased IL-10 and reduced IL-12p40 production by colonic DC. These effects appear most pronounced in patients with clinical improvement. Two patients with no clinical response after treatment with VSL#3 showed a less marked posttreatment reduction in IL-12p40 than did DC from VSL#3 responders (Fig. 5A, solid symbols). In addition, VSL#3 responders demonstrated an increased proportion of IL-10-positive DC not observed in nonresponders. The data suggest an association between change toward beneficial DC cytokine profile and clinical efficacy.

We also compared cytokine changes in all patients (VSL#3-treated or placebo) who improved clinically posttreatment with those who did not. There were no significant differences in any measured parameter between the groups; changes in DC profile may therefore relate to effects of treatment rather than to disease resolution alone. There were also no differences between patients in the placebo-treated group who achieved remission and those who had no clinical response in DC changes for surface markers or cytokines.

There was a lack of a clear relationship in those who improved clinically after 8 weeks of VSL#3 between the severity of histological inflammation and changes in DC function and cytokines. It is well recognized that histological change correlates poorly with clinical state, and that histological improvement lags substantially behind clinical
improvement. Presumably, as clinical improvement ensues the deactivation of inflammatory cells and pathways occurs before the tissue clearance of such cells. Earlier work has shown that clinical remission in UC may not be accompanied by endoscopic remission and uncommonly by mucosal healing.38 Solem et al39 showed that there is lack of association between inflammatory markers or endoscopic activity with histological inflammation in patients with UC.

Recent studies have identified IL-23 as a mediator of intestinal inflammation and IL-23 shares the p40 subunit with IL-12.40,41 IL-23 is produced by activated myeloid cells including DC following bacterial stimulation 42 or via CD40 signaling.41 Our assay for intracellular cytokine does not currently allow us to distinguish whether IL-12p40 reflects biologically active IL-12 or IL-23 but the effects of probiotic bacteria on DC IL-12p40 production may impact IL-23 production.

In patients with pouchitis, VSL#3 increases pouch tissue IL-10 and reduces proinflammatory cytokines IL1-α, IFN-γ, and TNF-α to untreated levels. These beneficial immune changes provide at least a partial explanation for the efficacy of VSL#3 in pouchitis.43 Probiotics may also have an indirect effect via antigen presenting cells in the gut; human monocyte-derived DC matured in the presence of Lactobacillus rhamnosus resulted in both reduced T-cell proliferation and IL-2, IL-4, and IL-10 production.44 Several studies have demonstrated that therapies for IBD modulate DC functions. We have previously shown that CD patients treated with anti-TNF-α antibody (infliximab) demonstrated downregulation of CD40 expression on colonic DC.15 Thomas et al45 recently showed that Saccharomyces bourladii, a probiotic yeast preparation, may exhibit part of its antiinflammatory activity through modulation of DC phenotype function and migration. In vitro culture of human monocyte-derived myeloid DC with S. bourladii supernatants led to reduced expression of CD40, CD80, and CCR7 on DC, increased IL-10, and reduced IL-6 and TNF-α. In mice Lactobacillus acidophilus strain L-92 induces apoptosis of antigen-stimulated T cells by modulating the DC function in vitro and in vivo.46 Furthermore, Mannon et al47 have demonstrated that the clinical benefit from granulocyte-colony stimulating factor (G-CSF) treatment in CD is accompanied by an increase in lamina propria plasmacytoid DC (CD123+), a DC phenotype associated with regulatory responses; this increase was restricted only to patients who had a clinical response.

Increased concentrations of both total fecal microbiota and bifidobacteria is associated with increased IL-10 by colonic DC, an observation consistent with effects of

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Pre Median (Range)</th>
<th>Post Median (Range)</th>
<th>Change Median (Range)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ</td>
<td>0 (0, 90)</td>
<td>2 (0, 51)</td>
<td>0 (−77, 17)</td>
<td>0.96</td>
</tr>
<tr>
<td>IL-2</td>
<td>0 (0, 20)</td>
<td>1 (0, 81)</td>
<td>1 (0, 60)</td>
<td>0.12</td>
</tr>
<tr>
<td>IL-10</td>
<td>6 (0, 259)</td>
<td>3 (0, 142)</td>
<td>−3 (−116, 48)</td>
<td>0.32</td>
</tr>
<tr>
<td>IL-8</td>
<td>2357 (182, 3991)</td>
<td>3252 (1063, 7439)</td>
<td>1872 (−2676, 4482)</td>
<td>0.14</td>
</tr>
<tr>
<td>IL-6</td>
<td>392 (3, 1861)</td>
<td>577 (54, 3757)</td>
<td>144 (−447, 3358)</td>
<td>0.37</td>
</tr>
<tr>
<td>IL-4</td>
<td>0.3 (0, 23)</td>
<td>3 (0, 79)</td>
<td>2 (−9, 56)</td>
<td>0.19</td>
</tr>
<tr>
<td>IL-5</td>
<td>3 (1, 9)</td>
<td>6 (0, 70)</td>
<td>1 (−4, 60)</td>
<td>0.26</td>
</tr>
<tr>
<td>IL-1beta</td>
<td>3 (0, 11)</td>
<td>3 (1, 16)</td>
<td>1 (−2, 5)</td>
<td>0.14</td>
</tr>
<tr>
<td>TNF-α</td>
<td>3 (0, 5)</td>
<td>6 (0, 50)</td>
<td>4 (−2, 47)</td>
<td>0.06</td>
</tr>
<tr>
<td>IL-12p70</td>
<td>5 (1, 8)</td>
<td>8 (2, 24)</td>
<td>3 (−4, 23)</td>
<td>0.06</td>
</tr>
</tbody>
</table>
bifidobacteria on DC observed in vitro. Treatment of patients with CD with prebiotic fructo-oligosaccharide increases total gut bacteria and bifidobacteria in patients with clinical improvement, together with a trend toward increase IL-10+DC.

Corticosteroids have been known to affect DC function. Human monocyte-derived DC cultured with dexamethasone showed a higher endocytotic activity, a lower antigen presentation cell function, and a lower capacity to secrete cytokines than untreated cells. In vitro steroids inhibit DC production of IL-12p70 and TNF-α, and corticosteroid-exposed DC generate IL-10 producing regulatory T cells. Here we demonstrate for the first time in a defined group of patients with acute UC, the beneficial effects of oral steroid therapy on ex vivo colonic DC suggesting that one mechanism by which corticosteroids ameliorates intestinal inflammation is via modulation of DC. Corticosteroids had similar effects to those of VSL#3 on DC function in vivo.

Changes in DC with therapy may be from sampling of bacteria by DC or via bacterial interactions with other cells. It is unlikely to be secondary to resolution of inflammation, as beneficial cytokine changes by DC were seen even when posttreatment tissue still contained inflammatory cells by histological criteria.

Inflammatory cell infiltrates are slow to resolve after treatment of acute colitis, despite symptomatic and macroscopic mucosal improvement, and clinical remission, reinforcing the potential for longer-term effects on overt inflammation of changes in DC.

In conclusion, we showed the effects of medical therapy on DC function in acute UC, providing evidence that exogenously administered bacteria influence gut inflammatory activity via effects on gut DC. Clinical improvement in patients treated with VSL#3 but not placebo was associated with a significant increase in ongoing production of IL-10 and a decrease in IL-12p40 and TLR-2 expression in gut DC. Corticosteroids had similar effects. Accumulating data suggest that properties of DC may be fundamental to modulation of inflammation in IBD.

ACKNOWLEDGMENT

We thank VSL#3 Pharma for providing the study medication and Paul Bassett for statistical input.

REFERENCES

51. Woltman AM, de Fijter JW, Kamerling SW, et al. The effect of calci-
学霸图书馆（www.xuebalib.com）是一个“整合众多图书馆数据库资源，提供一站式文献检索和下载服务”的24小时在线不限IP图书馆。

图书馆致力于便利、促进学习与科研，提供最强文献下载服务。

图书馆导航：

图书馆首页 文献云下载 图书馆入口 外文数据库大全 疑难文献辅助工具