

Immunologic Alterations Associated With Oral Delivery of Anti-CD3 (OKT3) Monoclonal Antibodies in Patients With Moderate-to-Severe Ulcerative Colitis

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Aim: The aim of this study was to determine the immunologic effects and safety of oral anti-CD3 in patients with ulcerative colitis (UC).

Methods: An open-label pilot study of orally delivered anti-CD3 was performed in patients with moderate-to-severe UC. The primary end points were changes in immunologic parameters and evaluation for safety.

Results: Six subjects received oral OKT3. Biologic effects of oral anti-CD3 included significantly increased proliferation in response to anti-CD3 and anti-inflammatory gene expression profile in peripheral blood mononuclear cells. No serious treatment-related adverse events occurred.

Conclusion: Orally delivered anti-CD3 resulted in immunologic changes in patients with UC.

Key Words: ulcerative colitis, muromonab-CD3, regulatory T cells

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INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory disease of the colon with a relapsing and remitting course.^{1,2} Data from animal models and human studies suggest that inflammation occurs secondary to an inappropriate immune response to the commensal bacteria flora in genetically susceptible individuals.¹ In addition to exuberant effector T-cell activation, reduced function of immunomodulatory regulatory T cells (Tregs) has been proposed to contribute to the loss of mucosal homeostasis in UC.³ Consequently, therapeutic agents that modulate the T-cell responses are of significant interest for the treatment of UC.

Muromonab-CD3 (Orthoclone OKT3, "OKT3," Ortho Biotech, Bridgewater, NJ) is a murine antihuman IgG_{2a} monoclonal antibody that recognizes the T3 antigen complex (CD3) on human T lymphocytes.⁴ Intravenously administered OKT3 is effective for the treatment of acute allograft rejection after transplantation⁵⁻⁷ and modulates T-cell immunity by multiple mechanisms, including partial depletion of CD3⁺ lymphocytes, Treg induction, and altered T-cell receptor signaling.⁸ Clinical use of parenteral OKT3 has been limited by significant adverse reactions including cytokine release syndrome, a life-threatening reaction resulting from OKT3 crosslinking.⁸⁻¹¹ However, despite modifications that reduced this toxicity, the intravenously administered anti-CD3 antibody, visilizumab, was not effective at inducing remission in corticosteroid-resistant severe UC.¹²

Oral administration of anti-CD3 monoclonal antibody is a novel approach to induce an anti-inflammatory immune response *in vivo*. Previous studies have demonstrated that orally delivered anti-CD3 antibody is effective at ameliorating inflammation in a variety of murine models of autoimmunity including colitis.^{13–18} Oral anti-CD3 is taken up by gut-associated lymphoid tissue^{13,14} where it is associated with the induction of Tregs that reduce disease severity in a transforming growth factor β ^{13–17} or interleukin (IL)-10-dependent manner.¹⁸ Oral OKT3 is well tolerated in healthy human subjects and is associated with increased T-cell proliferation, transforming growth factor β -expressing Th3 cells, and increased FOXP3 expression in Tregs.¹⁹ To assess the biologic effects and safety of orally delivered OKT3 in inflammatory bowel disease, we conducted an open-label pilot study in patients with moderate-to-severe UC.

MATERIALS AND METHODS

Patients

This open-label pilot study was conducted at 2 sites in Boston, MA, between April 2011 and September 2012, following institutional review board approval (Partners Healthcare IRB 2009P001448, FDA IND 105882, ClinicalTrials.gov number NCT01287195). All patients gave written informed consent.

Eligible patients were 18–65 years of age with a diagnosis of UC for at least 3 months before enrollment, confirmed by endoscopy and histology, who had failed or not tolerated at least 1 conventional medication for UC. Patients were required to have moderate-to-severe disease activity at screening (Mayo score of 6–12, including an endoscopy subscore of ≥ 1).^{20,21}

Patients with a previous ileostomy, proctocolectomy, or subtotal colectomy with an ileorectal anastomosis, a history of colorectal neoplasia or dysplasia, malignancy within the preceding 5 years, autoimmune disease, active medically significant infections including tuberculosis or positive tuberculin testing, positive *Clostridium difficile* testing within 10 days before enrollment, and seropositivity for human immunodeficiency virus or hepatitis B virus surface antigen were excluded. Pregnant women and nursing mothers were excluded. Hospitalized patients and patients who required immediate surgical, endoscopic, or radiologic intervention for toxic megacolon, massive hemorrhage, perforation, infectious complications, or sepsis were excluded. Exclusion criteria also included previous parenteral nutrition, prior exposure to OKT3, known sensitivity to the study drug or Omeprazole, anti-mouse antibody titer $> 1:1000$, and participation in another clinical trial within 30 days. Additional exclusion criteria included serum creatinine ≥ 2.0 mg/dL; bilirubin, alkaline phosphatase, AST or ALT $\geq 1.5 \times$ upper limit of normal; hemoglobin < 10.5 g/dL, platelets $< 100 \times 10^3/\mu\text{L}$, white cell count

$< 0.7 \times 10^3/\mu\text{L}$, or IgG anti-cardiolipin antibody > 16 international units.

Concomitant Medical Therapies

Patients were allowed to remain on oral or rectal 5-aminosalicylate medications and oral corticosteroids provided they were prescribed at stable doses for at least 4 weeks before enrollment. Anti-tumor necrosis factor biologics, immunomodulators, and rectal corticosteroids were discontinued at least 4 weeks before entry. Nonsteroidal anti-inflammatories and aspirin were discontinued at least 10 days before entry.

Study Design

This was an open-label pilot phase 1b/2a clinical trial. Muromonab-CD3 (OKT3) was purchased from Ortho Biotech. Patients received 1-mg OKT3 once daily by mouth for 30 days. Dosing occurred in the morning before breakfast, following an 8-hour fast. To protect OKT3 from degradation in the stomach, Omeprazole 20 mg was started 48 hours before the study drug and administered once daily orally, 20 minutes before OKT3 ingestion. The first 3 patients were monitored in the hospital overnight after receiving the first dose of OKT3. Subsequent patients received the first dose of study drug in the clinic and were observed for 4 hours after dosing.

The study was originally designed as a dose-escalation study to determine the safety and biologic response of 1 and 2 mg of oral OKT3 in patients with moderate-to-severe UC with a total planned recruitment was 16 patients. However, the study was terminated after enrolling 6 patients because of lack of access to the study drug, as the manufacturer discontinued sales of OKT3 during study recruitment.

Immunologic Assessments

Whole blood and serum were obtained at each study visit. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood by density gradient centrifugation over Ficoll-Paque Plus (GE Healthcare, Uppsala, Sweden). PBMCs were either used fresh or frozen in fetal calf serum supplemented with 10% dimethyl sulfoxide and stored for later use, as indicated.

Proliferation Assays

Fresh PBMCs were cultured at 2×10^5 cells per well, in triplicate, in RPMI 1640 medium supplemented with 5% fetal bovine serum (Gibco, Grand Island, NY) at 37°C/5% CO₂ in the presence of 5 $\mu\text{g}/\text{mL}$ anti-CD3 antibody (clone OKT3, eBioscience, San Diego, CA). One millicurie tritiated thymidine was added to each well 18 hours before harvesting. Cells were harvested at 66 hours, and proliferation was measured via scintillation counting.

Cytokine Analysis

Supernatants were collected from proliferation assays after 48 hours and were frozen at -80°C . Supernatants were thawed and analyzed by bead-based multi-analyte profiling according to the manufacturer's instructions (Luminex, R&D Systems, Minneapolis, MN).

FACS Analysis

Frozen PBMCs were thawed and stained with a panel of fluorochrome-conjugated antibodies against multiple surface markers for 30 minutes at 4°C (Supplementary Table S1). For intracellular staining, cells were then fixed and permeabilized (FOXP3 staining buffer set, eBioscience), followed by intracellular staining with fluorochrome-conjugated antibodies for 60 minutes at 4°C . Events were collected on a BD FACS Aria II flow cytometer (BD Biosciences, San Diego, CA) and were analyzed using FlowJo 10.6 (FlowJo, Ashland, OR). Samples were batched by subject for staining and analysis.

Analysis of Gene Expression

Frozen PBMCs were thawed, stained as above, and sorted on a BD FACS Aria II (BD Biosciences) into $\text{CD4}^+\text{CD25}^{\text{hi}}\text{CD127}^{\text{lo}}$ Tregs, $\text{CD4}^+\text{CD25}^{\text{lo-int}}\text{CD127}^{\text{hi}}$ conventional T cells (Tcons), and $\text{CD14}^+\text{CD16}^-$ classical monocytes. Sorted cells were suspended in RLT buffer (Qiagen, Hilden, Germany). Expression of immune-related genes was quantified using the nCounter Human Immunology v2 gene expression codeset (Nanostring, Seattle, WA) according to the manufacturer's instructions. This codeset determines expression of 579 immune-related transcripts, controlling for technical variation with 14 spiked-in positive and negative controls, and for RNA content with 15 internal reference genes. Gene expression above background was taken to be ≥ 30 transcripts per lane. Gene ontology and pathway analysis were performed using Ingenuity pathway analysis (Qiagen, Redwood City, CA). The nCounter Human Immunology v2 gene list was set as the background gene list for Ingenuity pathway analysis.

Clinical Evaluations

All patients underwent a medical history, physical examination, evaluation of adverse events (AEs), and blood tests, including complete blood count, comprehensive metabolic panel, erythrocyte sedimentation rate, C-reactive protein (CRP), and anti-mouse antibodies at baseline (day 0), day 1, day 2, week 1, week 3, week 5, and week 10. Patients underwent a flexible sigmoidoscopy at screening and week 5 to assess mucosal disease activity.

AEs were coded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03.²² All AEs were reviewed by an independent data safety monitoring board.

The Mayo score was obtained at baseline and week 5.^{20,21} The 9-point partial Mayo score²³ and the Simple Clinical Colitis

Activity Index²⁴ (SCCAI) were obtained baseline and weeks 1, 3, and 5. Clinical response was defined as a decrease in the Mayo score from baseline of at least 3 points and at least 30%, with an accompanying decrease in the rectal bleeding subscore of at least 1 point or an absolute rectal bleeding subscore of 0 or 1.^{20,21} Clinical remission was defined as a total Mayo score of 2 or less with no individual subscore of greater than 1.^{20,21} Mucosal healing was defined as an endoscopic subscore of 0 or 1.^{20,21}

Mucosal biopsies were obtained from the most inflamed area seen during flexible sigmoidoscopy and blindly scored by a single pathologist (A.K.B.). The histologic scoring system is defined in Supplementary Table S2.

Statistical Analysis

Statistical testing was performed using GraphPad Prism 5.0f (GraphPad Software, San Diego, CA). Parametric data were compared using Student *t* test. Nonparametric data were compared using the Mann–Whitney *U* test. Spearman rank correlation was performed for nonparametric data. A *P* value of <0.05 was considered significant. nSolver 2.6 (Nanostring) was used to normalize gene expression data and to determine fold changes in gene expression compared with baseline. nSolver used the Benjamini–Hochberg–Yekutieli procedure to control the false discovery rate.

RESULTS

Subject Enrollment

A total of 8 patients were screened for the study, and 6 patients met the criteria for enrollment, all of whom received OKT3. All 6 patients completed OKT3 therapy until week 3, whereas 4 patients completed the full 30-day course of OKT3 with assessment at week 5. One patient withdrew on day 23 of treatment and sought alternative medical therapy for worsening of UC. A second patient, who had a clinical response to therapy at week 3, withdrew for reasons unrelated to the study.

Patient Baseline Characteristics

Five patients were male, and 1 patient was female, with a mean age of 39.5 ± 13.5 years (mean \pm standard deviation) and a mean disease duration of 7.5 ± 4.5 years. Mean Mayo score at enrollment was 8.8 ± 1.6 , partial Mayo score was 6.2 ± 1.3 , and SCCAI was 7.5 ± 1.6 . Two patients were on oral prednisone (7.5 and 10 mg) at baseline. No patients were on 5-aminosalicylates, immunomodulators, or biologics. Baseline characteristics of the study subjects are given in Table 1.

Effect of Oral OKT3 on T-Cell Proliferation and Cytokine Production

Oral OKT3 treatment in healthy controls has been shown to increase the *in vitro* proliferative responses of T cells to

TABLE 1. Demographic and Clinical Characteristics at Baseline

Study ID	Gender	Age (y)	Disease Duration (y)	Disease Extent (y)	Concomitant IBD Medications	Mayo Score	SCCAI	Hb (g/dL)	HCT (%)	WCC ($\times 10^3/mm^3$)	Albumin (g/dL)	ESR (mm/h)	CRP (mg/dL)
1	Female	65.4	14.8	Pancolitis	Nil	9	9	14.1	42.4	9.3	4.1	19	2.3
2	Male	29.6	1.8	Pancolitis	Prednisone 10 mg	10	7	11.2	32.2	11.4	4	1	2.7
3	Male	29.9	6.3	Pancolitis	Nil	6	6	9.6	31.8	6.1	4	63	26.3
4	Male	34.4	9.5	Left sided	Prednisone 7.5 mg	10	6	15.5	44.7	11.4	4	18	16.4
5	Male	42.1	4.2	Left sided	Nil	8	7	14.2	41.5	5.5	4.5	7	2.8
6	Male	35.8	8.5	Left sided	Nil	10	10	13.2	42.2	7.2	4.6	30	14.2

Reference ranges: Hb 13.5–17.5 g/dL, HCT 41%–53%, WCC $4.5\text{--}11.0 \times 10^3/mm^3$, albumin 3.5–5.0 g/dL, ESR 0–11 mm/h, CRP < 8 mg/dL. CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; Hb, hemoglobin; HCT, hematocrit; IBD, inflammatory bowel disease; SCCAI, Simple Clinical Colitis Activity Index; WCC, white cell count.

anti-CD3 antibody.¹⁹ In patients with UC, oral OKT3 therapy was associated with a significant increase in PB T-cell proliferation in response to anti-CD3 stimulation in vitro at week 1 ($P < 0.05$) (Fig. 1). Proliferation returned to baseline levels by week 5, after the study drug was discontinued. The supernatants from in vitro stimulated PBMCs were analyzed for cytokine expression. No statistically significant changes in expression of Th1-related (IFN- γ), Th17-related (IL-17A), proinflammatory (IL-1 β , IL-6, tumor necrosis factor) cytokines or IL-10 were seen (data not shown). IL-2, IL-4, IL-5, and IL-12 were not detected in culture supernatants.

Effect of Oral OKT3 on Immune Cell Subsets

As parenteral OKT3 treatment is known to be associated with CD3⁺ lymphocyte depletion,⁸ we determined whether a reduction in lymphocytes occurred in UC patients treated with oral OKT3. No statistically significant changes in total white cell count or lymphocyte count were seen with oral OKT3 therapy (data not shown). To determine whether oral OKT3 treatment was associated with changes in CD3 expression, pro- or anti-inflammatory immune cell subsets, FACS-based immunophenotyping was performed on PBMCs at baseline and weeks 1, 3, and 5. There was no depletion of CD3⁺ T cells seen during therapy (Supplementary Fig. 1A), and there was no downregulation of CD3⁺ expression, as determined by CD3⁺ mean fluorescence intensity (data not shown). There was no change in the percentage of CD4⁺ or CD8⁺ T cells, or in their naive or memory subsets, or in the CD4/CD8 T-cell ratio during treatment with oral OKT3 (data not shown). There was no change in the percentage of CD4⁺CD45RA⁻ memory T cells expressing T helper (Th) lineage markers CXCR3 (Th1), CD294 (Th2), and CD161 (Th17) (data not shown). Furthermore, an increase in CD4⁺CD25^{hi}CD127^{lo} Tregs or CD4⁺LAP⁺ T cells was not observed (Supplementary Fig. 1B, C). Moreover, oral OKT3 therapy was not associated with a change in expression of molecules associated with Treg function, including FOXP3, CTLA-4, CD39, or the transcription factor HELIOS (data not shown). There were additionally no significant changes in the

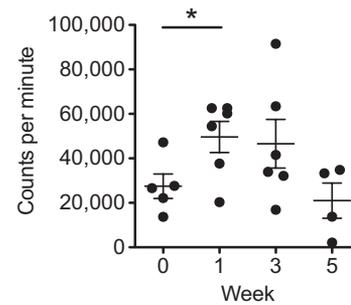


FIGURE 1. In vitro T-cell proliferation assessed by thymidine incorporation (counts per minute) in anti-CD3-stimulated peripheral blood mononuclear cells obtained from patients at weeks 0, 1, 3, and 5. * denotes $P < 0.05$.

proportion of CD19⁺ B cells, CD3⁻CD19⁻CD14⁺CD16⁻ classical monocytes, or CD3⁻CD19⁻HLA-DR⁺CD11c⁺ myeloid dendritic cells (data not shown).

Effect of Oral OKT3 on Gene Expression in PB Tcons and Monocytes

To determine whether oral OKT3 therapy was associated with a change in gene expression among T cells and myeloid cells, immune-related gene expression was evaluated in sorted PB Tcons and CD14⁺ classical monocytes, using the Nanostring platform. Very low cell numbers precluded analysis of gene expression in sorted Tregs. Normalized gene expression in sorted Tcons and CD14⁺ monocytes correlated well with gene expression in previously published data sets using the same platform (data not shown).^{25,26}

Sorted Tcons from baseline, weeks 1 and 3 were available from 4 patients. At baseline, 228/579 (39.4%) transcripts were expressed above background. This proportion remained unchanged at weeks 1 and 3. Consistent with cellular origin, 9 of the most highly expressed transcripts were associated with T-cell receptor signaling, whereas 3 were associated with cell adhesion. Only 4 transcripts were upregulated ≥ 2 -fold at week 1 of treatment: *KLRB1* (encoding CD161), *GZMK* (granzyme K), *KLRG1* (killer cell lectin-like receptor 1), and *CD45RO*. Six transcripts were downregulated > 2 -fold at week 1 of treatment. *TLR2* (Toll-like receptor 2) was downregulated in all 4 donors. *IL32* and *IFIT2* (interferon-induced protein with tetratricopeptide repeats 2) were downregulated in 3/4 donors. *NCAM1* (neural cell adhesion molecule 1 [CD56]), *LILRA3* (leukocyte immunoglobulin-like receptor subfamily A member 3), and *HLA-DRA* were downregulated in 2/4 donors.

Sorted CD14⁺ monocytes were available from 6 patients at baseline, week 1, and week 3. No transcripts were upregulated with treatment ≥ 2 -fold at any time. Transcripts for *IL8* (CXCL8) and the costimulatory molecule *CD83* were downregulated ≥ 2 -fold at week 1.

Safety

All 6 patients received at least 3 weeks of oral OKT3, and 4 patients received 30 days of drug therapy. Oral administration of OKT3 was well tolerated by all patients. No patients developed symptoms or signs consistent with cytokine release syndrome during the initial dose administration or follow-up. There were no clinically significant changes in hematological or biochemical parameters, and no patients developed anti-mouse antibodies throughout the 10-week treatment and follow-up period. One patient reported mild periorbital edema for 2 weeks while on the study drug (CTCAE grade 1). One patient developed a perianal abscess 1 week after finishing the planned treatment period, necessitating parenteral antibiotics (CTCAE grade 3). This patient subsequently developed a perianal fistula, and the patient's diagnosis was later changed to

Crohn disease. One patient experienced worsening of UC and withdrew consent after 3 weeks to seek alternative therapy. The subject was admitted 1 week later to the hospital for parenteral corticosteroids.

Clinical and Endoscopic Assessment

Mayo scores at baseline and week 5 were 8.8 ± 1.6 and 7.5 ± 1.7 ($P = 0.25$; [Supplementary Fig. 1C](#)). Four patients were evaluable at week 5. Of these, 2 achieved a clinical response at that time. Mucosal healing was seen in 1 patient. Partial Mayo scores showed a trend toward improvement at week 3. These were 6.2 ± 1.3 , 3.8 ± 2.2 ($P = 0.052$ vs baseline), and 5.3 ± 1.3 ($P = 0.35$ vs baseline) at weeks 0, 3, and 5, respectively ([Supplementary Fig. 1D](#)). Based on the partial Mayo score, 3 patients (50%) had a clinical response at week 3, including 1 patient who achieved a clinical remission. Histologic classification improved in one patient from "severe" at week 0 to "mild" at week 5. Histologic appearance was stable in the other 3 evaluable patients ([Supplementary Fig. 1E](#)). No statistically significant changes were observed in erythrocyte sedimentation rate or CRP (data not shown).

DISCUSSION

This is the first study to report the effects of orally delivered anti-CD3 antibodies in patients with moderate-to-severe UC. In this open-label pilot study, 6 patients treated with oral OKT3 demonstrated modest changes in immunologic parameters including PBMC proliferation and gene expression profiles. There were no alarming safety signals identified including no evidence of cytokine release syndrome or development of human anti-mouse antibodies.

Immunologic changes were associated with delivery of OKT3, demonstrating biologic activity of anti-CD3 antibodies when delivered orally. An increase in ex vivo PBMC proliferation in response to T-cell stimulation was seen in patients during the first week of OKT3 administration, returning to baseline by week 5. This finding was also observed in a phase 1 study of oral OKT3 in healthy subjects.¹⁹ In addition, OKT3 use was associated with modulation in mRNA expression of markers associated with T-cell activation and terminal differentiation in PB Tcons during therapy, including the memory T-cell marker CD45RO, CD161 (encoded by *KLRB1*) expressed on cells with potential to differentiate into Th17 cells.²⁷ The gene expression data set additionally highlighted downregulation of proinflammatory gene expression in sorted PB Tcons including *TLR2*, *IL-32*, *HLA-DRA*, and *NCAM1* (CD56). Based on these data, we hypothesize that oral OKT3 administration is associated with T-cell activation within the intestine resulting in the observed hyperproliferation with downstream anti-inflammatory immune effects. However, the gene expression data from this study should be interpreted with caution due to the very small sample size.

We hypothesized that oral OKT3 delivery in UC would be associated with an induction of regulatory CD4⁺ subsets or a change in functional markers on Tregs. However, we did not observe this in our cohort. Given our small study size, it is possible that the study was underpowered to detect such differences. Alternatively, these findings may reflect recruitment of regulatory T cells out of the blood to the site of inflammation within the colon in patients with UC. Similarly, prevention of murine colitis with oral anti-CD3 was not associated with a change in the proportions or phenotype of CD4⁺ T cells or Tregs in the spleen or mesenteric lymph nodes.¹⁸ The effect of oral OKT3 on the mucosal immune system in patients with UC was not addressed in this study and deserves investigation in future studies.

This small pilot study was underpowered to adequately detect significant safety or efficacy signals associated with orally delivered OKT3. We did confirm that, in contrast to parenteral administration of OKT3, oral delivery was not associated with cytokine release syndrome, T-cell depletion, or generation of human anti-mouse antibodies in subjects with UC.^{8–11} The side effect profile in patients with active UC was similar to that seen in other human studies.^{19,28,29} These findings suggest that orally delivered OKT3 probably does not enter the blood in high concentrations.

Although placebo effect is likely, 3/6 subjects had a clinical response to oral OKT3 at week 3. However, no changes in CRP were observed during the study period, and only 1 patient showed endoscopic and histologic improvement at week 5. Even patients with a clinical response to oral OKT3 had an increase in partial Mayo score after the drug was discontinued. This suggests that short-term administration of oral OKT3 is unlikely to maintain remission beyond its delivery by sustainably altering immunologic function.

One of the key disadvantages of biologic drugs is their poor bioavailability via the oral route due to enzymatic degradation and unfolding in the gastrointestinal tract. This study demonstrates that oral OKT3 may overcome this challenge via immunomodulation that occurs in the intestinal mucosa with little systemic drug exposure. Accordingly, oral administration appears to avoid the significant safety concerns associated with parenteral OKT3 delivery including T-cell depletion and cytokine release syndrome. Although Orthoclone OKT3 is no longer actively marketed in the United States, alternative anti-CD3 antibodies will probably become available for future study. Larger studies are warranted to further elucidate the efficacy, safety, and mechanisms associated with oral anti-CD3 in patients with UC.

SUPPLEMENTARY DATA

Supplementary data are available at *Crohn's & Colitis 360* online.

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