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Dissecting Common and Unique Effects of Anti-α4β7 and Anti-Tumor Necrosis Factor Treatment in Ulcerative Colitis

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Abstract

Background and Aims: Vedolizumab is an anti-α4β7 antibody approved for the treatment of ulcerative colitis [UC]. Although it is assumed that vedolizumab blocks intestinal homing of lymphocytes, its effects on different intestinal cell populations are not fully stablished. In order to establish the unique mechanisms of action of vedolizumab in UC patients, we compared its effects to those induced by anti-tumour necrosis factor [TNF].

Methods: Patients with active UC [endoscopic Mayo score >1] starting vedolizumab [*n* = 33] or anti-TNF [*n* = 45] and controls [*n* = 22] were included. Colon biopsies [at weeks 0, 14 and 46] and blood samples [at weeks 0, 2, 6, 14, 30 and 46] were used for cell phenotyping, transcriptional analysis [qPCR], and to measure receptor occupancy.

Results: Vedolizumab, in contrast to anti-TNF, significantly reduced the proportion of $\alpha4\beta7^+$ cells within intestinal T subsets while preserving the percentage of $\alpha4\beta7^+$ plasma cells. The marked decrease in α4β7 did not change the percentage of colonic $\alpha E\beta7^+$ cells [at 46 weeks]. Both vedolizumab and anti-TNF significantly downregulated inflammation-related genes in the colon of responders [Mayo score < 2]. Moreover, both treatments significantly decreased the percentage of intestinal, but not blood, total lymphocytes [T and plasma cells], as well as the proportion of $\alpha4\beta1^+$ cells within intestinal T lymphocytes.

Conclusions: Our data show that while vedolizumab and anti-TNF block two unrelated targets, they induce remarkably similar effects. On the other hand, vedolizumab's unique mechanism of action relies on blocking intestinal trafficking of α4β7 T cells, despite effectively binding to B and plasma cells that express α4β7.

Key Words: Vedolizumab; inflammatory bowel disease; αEβ7; α4β1

1. Introduction

Ulcerative colitis [UC] is a chronic remitting and relapsing inflammatory bowel disease [IBD] that affects the large intestine and is characterized by superficial mucosal inflammation that can lead to diarrhoea and rectal bleeding. Available treatments include 5-aminosalicyaltes for mild flares and corticosteroids and immunosuppressants for moderate-to-severe disease. Patients with inadequate response to conventional therapies are eligible to receive biological therapy including anti-tumour necrosis factor [TNF] antibodies, anti-α4β7 or, more recently, a pan-Janus kinase inhibitor [tofacitinib].[1,2] While these options target seemingly independent pathways, they can all have potent anti-inflammatory effects and promote healing of the colonic mucosa in a proportion of patients.[3–6]

Anti-TNF blocking monoclonal antibodies [mAbs] interfere with the effects of TNF, a potent and pleiotropic cytokine that promotes phagocytosis, bacterial killing, leukocyte recruitment, antigen presentation and tissue remodelling. In addition, anti-TNF mAbs have been shown to bind to membrane-bound TNF and promote apoptosis of cells potentially involved in the inflammatory cascade.[7] In contrast to anti-TNF, vedolizumab, approved for the treatment of both UC and Crohn's disease, binds to the α4β7 integrin and blocks its binding to endothelial mucosal addressin cell adhesion molecule-1 [MAdCAM-1].[8,9] α4β7 is primarily expressed by certain T-cell subsets[10,11] and B cells,[12,13] including plasma cells,[14–16] although it can also be expressed in innate cells such as granulocytes, natural killer cells[8] and monocytes.[17] Studies in mice,[18] and later in primates,[19] have demonstrated the involvement of this receptor in the specific homing of memory T cells towards gut-associated lymphoid tissues. α4β7 binds to MAdCAM-1, a receptor that is highly upregulated in the intestinal vasculature of both UC and Crohn's disease patients[20] and that mediates leukocyte recruitment towards the lamina propria. In addition, α4β7 and MAdCAM-1-dependent interactions have been reported to support naïve T- and B-cell recruitment towards induction sites [i.e. Peyer patches and intestinal lymphoid aggregates].[21,22]

Despite the wide use of anti-TNF and anti-integrin therapy, the differential mechanisms of action underlying these drugs have not been completely characterized.

Here, we provide novel information on the effects that vedolizumab exerts on lymphoid cell circulation and accumulation in both the systemic and the colonic compartments of a group of UC patients receiving vedolizumab for up to 1 year. Our goal is to understand vedolizumab's mechanisms of action. By comparing with an independent cohort of patients receiving anti-TNF therapy we aim to differentiate between the specific, as well as non-specific, downstream effects of vedolizumab therapy.

2. Materials and methods

Additional details of the materials and methods used are given in the Supplementary Materials.

2.1 Study subjects and sample collection

Patients with an established diagnosis of UC of at least 3 months' duration, and non-IBD controls [Ctrl] were included in the study between May 31, 2017 and November 5, 2019. All patients signed a written informed consent. Controls were subjects undergoing a colonoscopy for mild gastrointestinal symptoms, or screening for colorectal cancer that presented no mucosal lesions. UC patients were those starting treatment with an anti-TNF antibody [infliximab, adalimumab or golimumab] or vedolizumab and followed-up for a maximum of 46 weeks. Patients underwent clinical and endoscopic evaluations at weeks 0, 14 and 46. All biopsy samples from controls and UC patients were obtained from the sigmoid colon [or rectum in the case of proctitis]. Follow-up samples were obtained whenever possible from the same locations. Blood was collected from UC patients at weeks 0, 2, 6, 14, 30 and 46. A total of 33 controls and 67 UC patients were recruited for this study. Whenever possible, both blood and biopsies were obtained from the same individuals. Patients who were switched to a different treatment due to lack of response or disease worsening dropped out of the study. Table S1 shows the clinical and demographic characteristics of the subjects included in the study stratified according to the type of sample and analysis. Patients were recruited at the Department of Gastroenterology, Hospital Clinic Barcelona, and the Hospital Universitari Mutua Terrassa. The study protocol was approved by the ethics committees at both centres.

2.2 Assessment of disease activity

Endoscopic UC activity at the time of the colonoscopy was assessed according to the Mayo endoscopic subscore.[23] Active UC was defined as a Mayo endoscopic subscore of >1. Endoscopic improvement was defined as a Mayo endoscopic subscore ≤1 at week 14 or 46.

2.3 Intestinal cell isolation

Colonic biopsies [n = 2–3 per patient] were collected from controls [n = 10] and UC patients [n = 47] and submitted to enzymatic digestion to obtain a cell suspension for flow analysis [Table S1, Group1].

2.4 Cell staining

Single-cell suspensions obtained from digested biopsies were stained with a Zombie Aqua Fixable Viability Kit [BioLegend] to exclude dead cells and with the following antibodies: CD20 FITC, CD8 FITC, α4[CD49d] PE [clone 9F10], CD3 PerCP, CD38 PerCP, CD4 PE-Cy7, α4[CD49d] PE-Cy7 [clone 9F10], β7 APC [clone FIB504], β1[CD29] APC-Cy7, αE[CD103] BV421, CD19 BV421 [all from Biolegend] and αE[CD103] PE (Becton Dickinson [BD]).

Fresh blood from controls [n = 20] and UC patients [n = 51] was collected into an EDTA K2 Vacutainer [BD] and immediately processed or kept in a rocking platform at 4°C [<30 min] until processing [Table S1, Group 3]. Two hundred microlitrers of blood was incubated for 15 min with Red Blood Cell Lysis Buffer [BioLegend] and washed twice with PBS. Cells were stained with CD8 FITC, CD19 FITC, β1[CD29] PE, CD38 PerCP, α4[CD49d] PE-Cy7 [clone 9F10], β7 APC [clone FIB504], CD45RA APC-Fire750, IgD APC-Cy7, αE[CD103] BV421 [BioLegend] and CD4 PerCP [BD].

Intestinal and blood samples were fixed using BD Stabilizing Fixative [BD], and Precision Count Beads [BioLegend] were then added. Samples were acquired using a BD FACSCanto II flow cytometer [BD] and analysed with FlowJO software [BD].

2.5 Occupancy assay

In brief, cells were co-stained with the anti-β7 PE [clone FIB540; BioLegend] antibody and vedolizumab labelled with Alexa Fluor 647.

2.6 RNA isolation

Biopsies [n = 8 controls and n = 58 patients] were placed in RNAlater RNA Stabilization Reagent [Qiagen] and stored at −80°C. Total RNA was extracted using a RNeasy Kit [Qiagen] according to the

manufacturer's instructions [Table S1, Group 2]. Blood [n = 14 patients] was collected in a PAXgene Blood RNA System [PreAnalytiX GmbH] and stored at –20°C. RNA was isolated using the PAXgene Blood RNA Kit [Qiagen] in a QIAcube instrument [Qiagen] following the manufacturer's instructions [Table S1, Group 2]. The purity and integrity of the total RNA were assessed with the 2100 Bioanalyzer [Agilent] and then quantified via a NanoDrop spectrophotometer [Nanodrop technologies]; only samples with an RNA integrity number [RIN] >7.0 were used.

2.7 Quantitative polymerase chain reaction

Total RNA was transcribed to cDNA using a reverse transcriptase [High Capacity cDNA Archive RT kit; Applied Biosystems]. For biopsy RNA, quantitative polymerase chain reaction [qPCR] was performed on selected genes including three endogenous controls [GAPDH, PPIC and UBA3] using custom-designed TaqMan Low Density Arrays [TLDA platform; Applied Biosystems]. For blood RNA, qPCR analysis was performed using a TaqMan Universal PCR Master Mix [Applied Biosystems] containing the probe of interest and β-actin [TaqMan primers and probes; Applied Biosystems]. Data are expressed as arbitrary units [AU], applying the 2^{-dCt} formula and correcting by the value of the endogenous genes.

2.8 Immunohistochemistry

Colonic biopsies were placed in 10% formalin for 24 h and then embedded in a paraffin block. Immunohistochemical staining of tissue sections was performed using commercially available rabbit anti-human CD103 antibody [1:1000; clone EPR4166(2); Abcam]. Image acquisition was performed on an Olympus BX51 microscope using CellF Software.

2.9 ELISA to measure vedolizumab levels

Serum was collected [Table S1, Group 4, n = 51] to determine vedolizumab concentration using a serum separator tube, and the sample was then stored at –80°C. The concentration of vedolizumab in serum samples was determined using the Promonitor-vedolizumab ELISA kit [Progenika] following the manufacturer's instructions.

2.10 Soluble α4β7 ELISA

A home-made ELISA was developed to measure levels of soluble α4β7 in serum [Group 4].

2.11 Statistical analysis

Graphs were made and statistical analysis was performed using Graphpad Prism [Graphpad Software]. Only data from available samples at the studied time point are included and provided as observed. Differences between groups were tested using the non-parametric Kruskal–Wallis test and correcting for multiple comparisons by controlling the false discovery rate [two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli]. Corrected p-values <0.05 were considered statistically significant.

3. Results

3.1 Treatment with vedolizumab achieves full occupancy of α4β7 on circulating and intestinal T and B lymphocytes

To quantify α4β7 occupancy in patients receiving vedolizumab, we set up an assay using in-house fluorochrome-conjugated vedolizumab together with a non-competing anti-β7 antibody and measured both vedolizumab-free α4β7 and total β7 expression independently of bound vedolizumab. Baseline α4β7 expression patterns, shown in Supplementary Figure 1 for the different blood and biopsy lymphocyte subsets, were in agreement with earlier studies.[10,11,14,24] We quantified receptor occupancy before and after treatment with vedolizumab in blood [Supplementary Figure 2], as well as in colonic biopsies [Figure 1]. In agreement with previous studies involving humans[25] and primates,[9,26] we found full occupancy [absence of free α4β7] in blood T and B cells at weeks 2, 6, 14, 30 and 46 of vedolizumab treatment [Supplementary Figure 2]. Vedolizumab serum concentrations were also measured and compared between responders and non-responders [Supplementary Figure 3]. In agreement with published data,[27] serum levels at an early time point [week 6] were significantly increased in responders compared to non-responders, while at the end of induction [weeks 14 and 46] no significant differences were observed. In colonic cells, occupancy was also 100% within $CD4^+$ T cells and $CD19^+$ lymphocytes [Figure 1]. In contrast, α4β7 was incompletely occupied [<99% occupancy] on intestinal $CD8^+$ T cells in 54% of patients at week 14 and in 71% of patients at week 46. Nonetheless, the presence of vedolizumab-free α4β7 on intestinal $CD8^+$ cells was not associated with lack of response to vedolizumab [shown in Figure 1B as open circles] nor was it significantly correlated with lower serum vedolizumab concentrations [p = 0.1603]. Indeed, the percentage of intestinal $CD8^+$ with unoccupied α4β7 was not significantly different between responders and non-responders at weeks 14 or 46 [data not shown].

3.2 Vedolizumab and anti-TNF treatment can reduce total lymphocytes in the colon of UC patients

At baseline, patients with active UC showed a significant increase in the percentage of $CD3^+CD8^+$ and $CD19^+CD38^+$ cells within the inflamed colonic lamina propria compared to healthy controls [Figure 2A and B]. Patients in the vedolizumab cohort [a majority of them refractory to anti-TNF] showed significantly higher percentages of $CD3^+CD4^+$ T cells at week 0 compared to controls [Figure 2A]. This percentage was significantly downregulated at weeks 14 [$p < 0.05$] and 46 [$p < 0.001$] of vedolizumab treatment compared to week 0, reaching at the final time point levels similar to those of healthy controls. In addition, treatment with either vedolizumab or anti-TNF induced a significant decrease, most pronounced at week 46, in the percentage of colonic $CD3^+CD8^+$ T cells and $CD19^+CD38^+$ plasma cells, taking into account all patients in the group regardless of treatment response [Figure 2A and B].

Despite their different mechanism of action, vedolizumab and anti-TNF induced similar changes in the expression of a number of genes upregulated in active UC. Indeed, we confirmed that endoscopic improvement [Mayo endoscopic score ≤ 1] was associated with a significant downregulation of a panel of genes related to innate [neutrophil, monocyte and macrophages] and adaptive [lymphocytes] inflammatory mediators [*CXCL9*, *CXCL10*, *FCGR3B*, *IL23A*, *IL17*, *IFNG]* in both anti-TNF- and vedolizumab-treated patients [Figure 2C].

In contrast to their effects within the intestinal mucosa, neither vedolizumab nor anti-TNF had any significant effect on the percentages of circulating $CD4^+$, $CD8^+$ or $CD19^+$ lymphocytes in peripheral blood up to 46 weeks after treatment initiation [Figure 3].

Overall, we observed that blocking either α4β7 or TNF led to reductions in the sizes of T- and plasma cell populations in the colon of UC patients without affecting circulating subsets. While

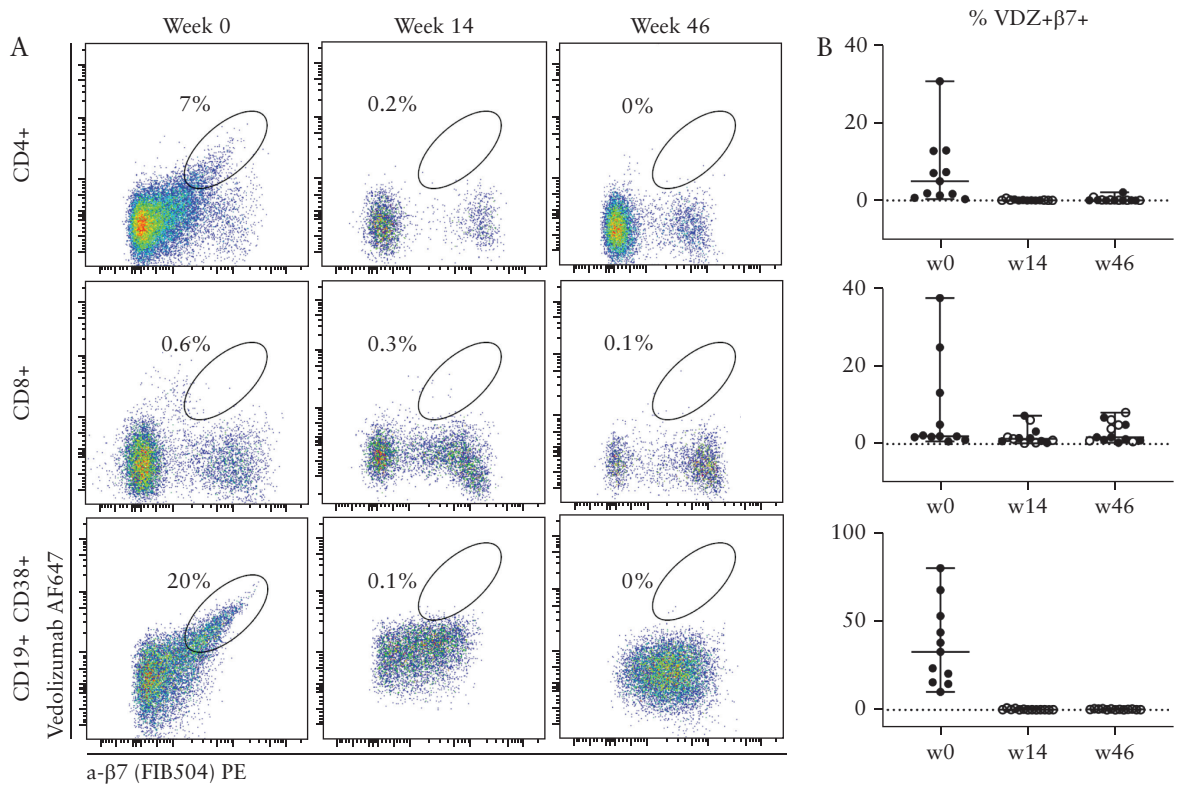

Figure 1. Treatment with vedolizumab results in high α4β7 occupancy within intestinal lymphocytes. Integrin occupancy was assessed by co-staining with fluorescently labelled vedolizumab and the non-competing anti-β7 mAb. [A] Flow cytometry plots of intestinal CD4⁺ and CD8⁺T cells, as well as of CD19⁺CD38⁺ plasma cells at weeks 0, 14 and 46 after initiation of vedolizumab [VDZ] treatment of a representative UC patient. Gates show cells with unoccupied α4β7 [VDZ⁺ β7⁺] and the percentage on each cell subset is depicted for a representative sample. [B] Percentage of unoccupied cells within the different lymphocyte populations at weeks 0, 14 and 46. Patients are represented as responders [closed circles] and non-responders [open circles] at weeks 14 or 46. Plot lines show median and range.

vedolizumab appears to have a more pronounced effect on the intestinal CD4⁺ T cell subset, this may also stem from the fact that the vedolizumab cohort, but not the anti-TNF cohort, included in our study presented an increased percentage of CD4⁺ T cells at baseline.

3.3 Vedolizumab specifically reduces intestinal α4β7⁺T cells and overall surface expression of α4β7 in blood

We next measured the effects of treatment on the proportions of α4β7⁺ cells within the different lymphocyte compartments. Vedolizumab significantly decreased the percentage of intestinal α4β7⁺ within the CD4⁺ and CD8⁺ T-cell compartments at weeks 14 and 46 of follow-up, taking into account all patients in the group regardless of response [Figure 4A]. In contrast, despite decreasing total CD8⁺ intestinal lymphocytes [Figure 2B], anti-TNF treatment did not change the percentage of α4β7⁺ cells within the CD8 compartment [Figure 4B].

The majority of plasma cells [CD19⁺CD38⁺] in the colon expressed high levels of α4β7 [Supplementary Figure 1 and Figure 4]. Nonetheless, and despite full receptor occupancy [Figure 1], the proportion of α4β7⁺ cells within the plasma cell compartment was not changed by vedolizumab or anti-TNF treatment [Figure 4]. These data demonstrate that while both treatments can reduce the total number of plasma cells in the intestinal mucosa, the proportion of those cells that express α4β7 remains the same for up to 46 weeks of follow-up.

In addition, vedolizumab, but not anti-TNF treatment, selectively increased the percentage of α4β7⁺ cells within memory CD4⁺CD45RA⁻ and CD19⁺IgD⁻ cells in the blood [Figure 5A] regardless of the response to treatment. This mild, but significant, increase was only detected at 1 year after initiation of the treatment. Nonetheless, in agreement with previous reports,[28] vedolizumab significantly decreased the surface expression of β7 [measured by a decrease in the mean fluorescence intensity of a non-competing anti-beta7 mAb] on all circulating memory T and B lymphocytes [Figure 5B]. The effect of vedolizumab on integrin surface expression was already detectable at week 2, the earliest time point studied. This was not true, however, in anti-TNF-treated patients, suggesting that vedolizumab exerts specific and fast-acting effects on α4β7 protein expression.

Vedolizumab-induced α4β7 internalization has been previously suggested,[29,30] and could indeed explain the reduction in β7 expression *in vivo*. To test this hypothesis, we incubated control peripheral blood mononuclear cells [PBMCs] with vedolizumab at different doses [0.5–50 μg/mL], or an anti-β7 antibody [clone FIB504; 0.5–10 μg/mL], and measured α4β7 expression 24 h later. Even concentrations of 50 μg/mL of vedolizumab [over therapeutic serum concentrations] did not induce receptor internalization [Supplementary Figure 4A]. In contrast, the anti-β7 antibody [clone FIB504] caused a robust dose-dependent internalization of α4β7 [Supplementary Figure 4A].[31]

In addition, when PBMCs from vedolizumab-treated patients were cultured *in vitro* they recovered expression of membrane α4β7

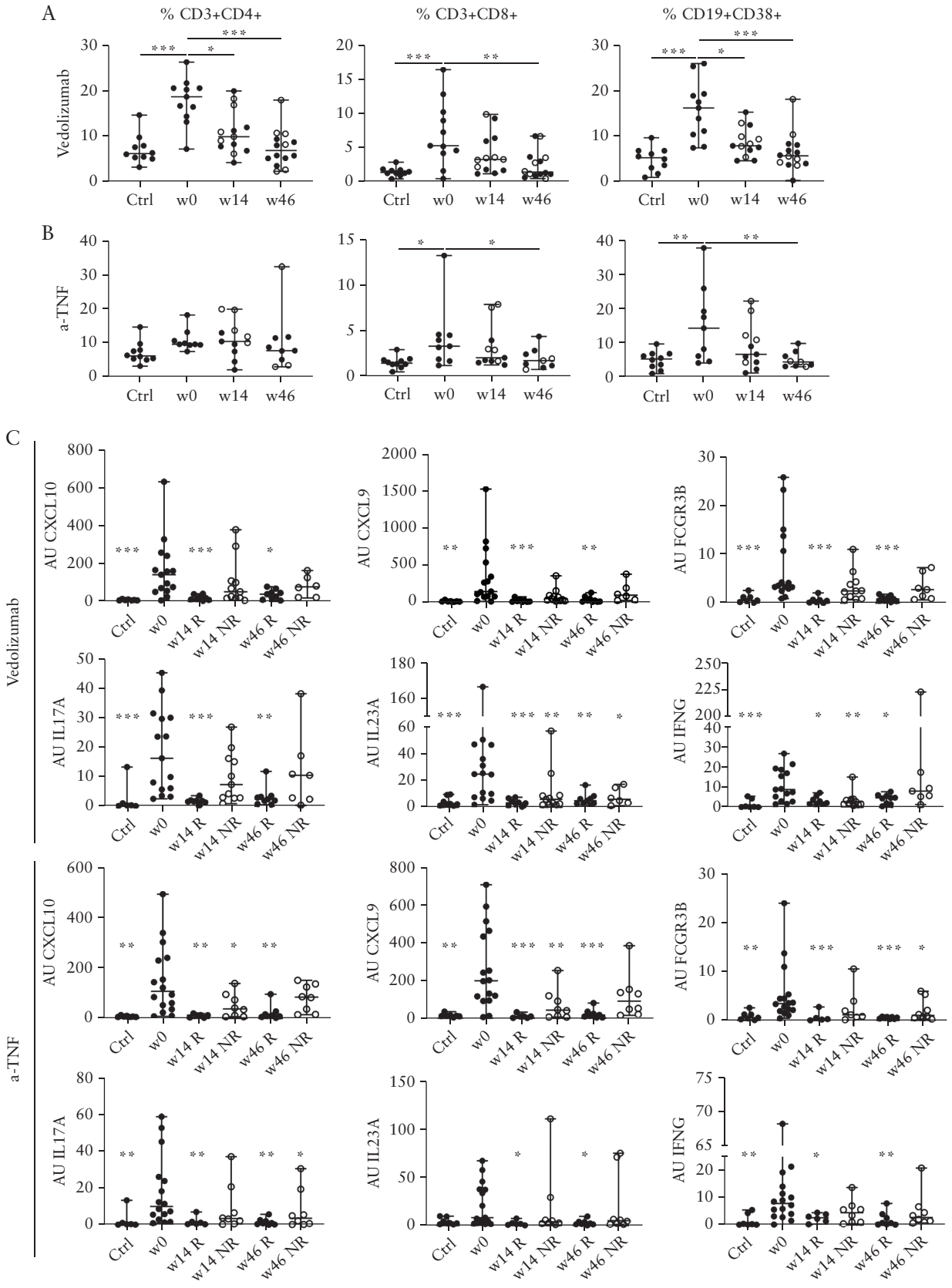

Figure 2. Vedolizumab and anti-TNF reduce total intestinal lymphocytes and decrease inflammatory gene expression. Percentages of intestinal $CD3^+CD4^+$, $CD3^+CD8^+$ and $CD19^+CD38^+$ in controls [Ctrl] and UC patients treated with [A] vedolizumab or [B] anti-TNF at weeks 0, 14 and 46. Patients are represented as responders [closed circles] and non-responders [open circles] at weeks 14 or 46. Scatter dot plot lines show median ± range taking into account responders and non-responders. [C] Gene expression in the colon is determined by qPCR for different inflammation markers in controls [Ctrl] and UC patients treated with vedolizumab or anti-TNF at weeks 0, 14 and 46. Differential gene expression analysis was performed for responders and non-responders separately. Data are expressed as arbitrary units [AU]. Bar graph lines show median ± range. False discovery rate-corrected *p*-values: *< 0.05; **< 0.01; and ***< 0.001.

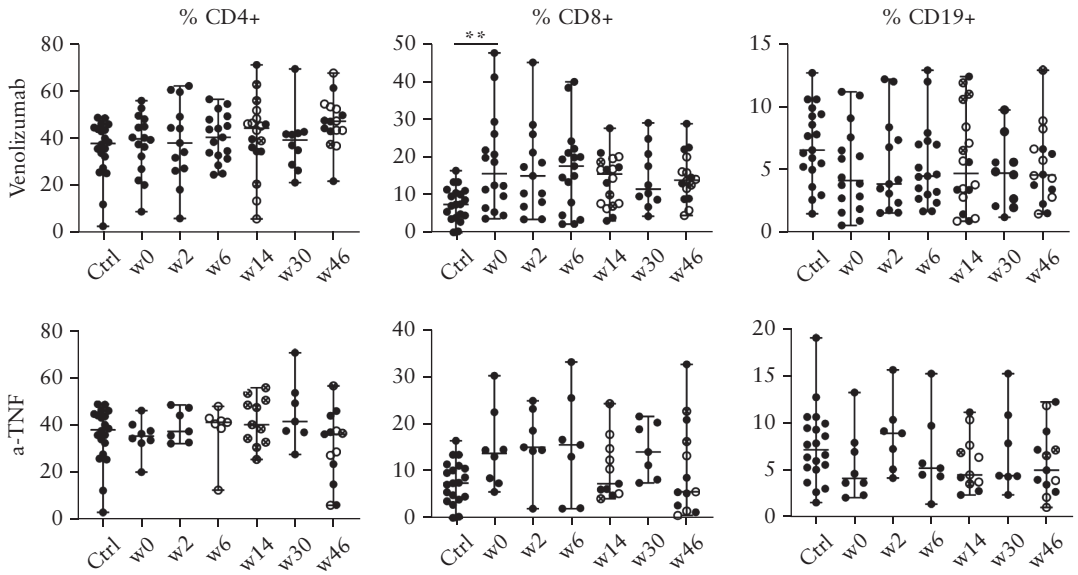

Figure 3. Vedolizumab and anti-TNF have no significant effect on peripheral blood total circulating lymphocytes. Percentages of circulating $CD3^+CD4^+$, $CD3^+CD8^+$ and $CD19^+CD38^+$ lymphocytes from total blood mononuclear cells in controls [Ctrl], UC patients treated with vedolizumab [A] or anti-TNF [B] at weeks 0, 2, 6, 14, 30 and 46. Patients are represented as responders [closed circles] and non-responders [open circles] at weeks 14 or 46. Scatter dot plot lines show median ± range taking into account responders and non-responders. False discovery rate-corrected *p*-value: **< 0.01.

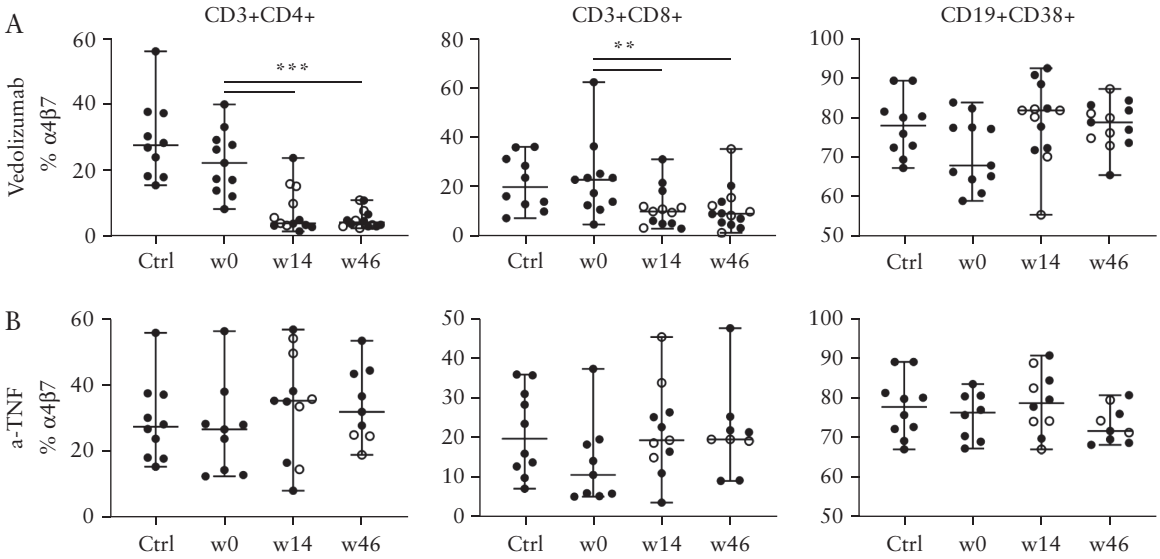

Figure 4. Vedolizumab decreases the frequency of α4β7 T cells but not plasma cells. Percentage of $\alpha4\beta7^+$ cells in $CD3^+CD4^+$, $CD3^+CD8^+$ and $CD19^+CD38^+$ intestinal lymphocytes determined by flow cytometry using co-staining with anti-α4 and anti-β7 antibodies. UC patients receiving vedolizumab [A] or anti-TNF [B] at weeks 0, 14 and 46. Patients are represented as responders [closed circles] and non-responders [open circles] at weeks 14 or 46. Plot lines show median ± range taking into account responders and non-responders. False discovery rate-corrected *p*-value: *< 0.05; **< 0.01; and ***< 0.001.

in a time-dependent manner [Figure 5C]. This new surface expression was prevented by cycloheximide [an inhibitor of protein synthesis], further suggesting that the integrin was not being recycled but rather synthesized *de novo* [Figure 5C]. We also tested the possibility of vedolizumab driving receptor shedding *in vivo*. In contrast to a recent study,[32] we could not detect soluble α4β7 in any of our samples [data not shown]. The test we used, however, had a detection limit of 26.9 ng/mL, which is higher than the concentrations previously reported [<15 ng/mL].[32] Thus, we cannot rule out a higher cleavage rate of the integrin as an explanation for the reduced density observed in the cellular surface.

Finally, we tested the hypothesis that vedolizumab may be decreasing the transcription of *ITGB7* and/or *ITGA4*, the genes encoding for the α4 and β7 chain. Vedolizumab had no significant effect on ITGB7 and ITGA4 transcription in whole blood RNA for up to 46 weeks of treatment [Supplementary Figure 4B].

3.4 Vedolizumab does not significantly change the percentage of mucosal $\alpha E\beta7^+$ lymphocytes

Expression of the αE [CD103] chain, which forms a heterodimer with the β7 chain, is acquired in mucosal surfaces in response to epithelial-derived transforming growth factor-β [TGF-β][33]. Indeed,

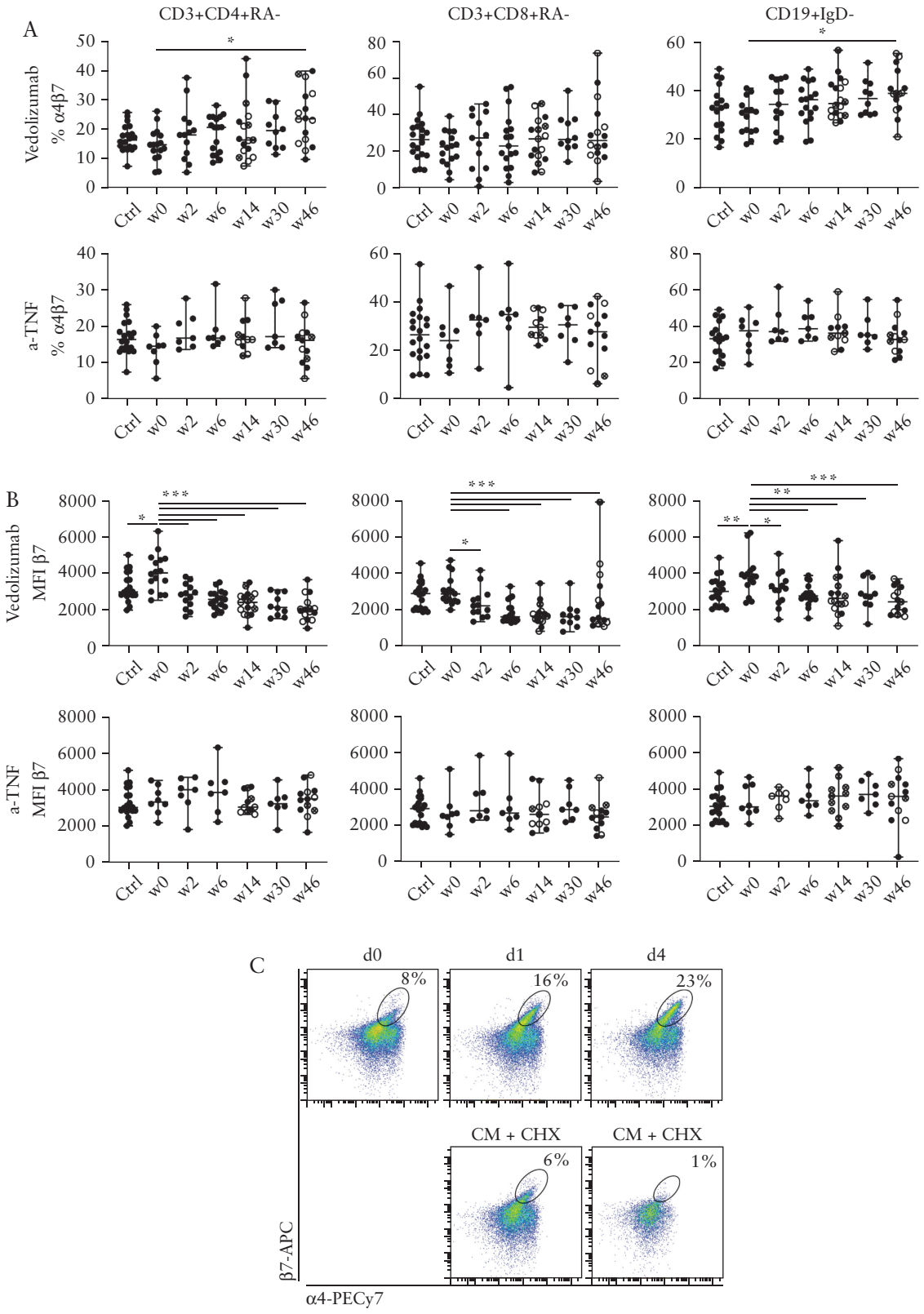

Figure 5. Vedolizumab induces changes in the expression of α4β7 in blood lymphocytes. [A] Percentage of α4β7$^+$ CD4$^+$ and CD8$^+$ memory [CD45RA$^-$] bloodT and B [CD19$^+$IgD$^-$] cells determined by flow cytometry using co-staining with anti-α4 and anti-β7 antibodies. [B] Mean fluorescence intensity [MFI] for β7 is shown for memory bloodT and B cells. For [A] and [B] the top row shows UC patients receiving vedolizumab at weeks 0, 2, 6, 14, 30 and 46; bottom row shows UC patients receiving anti-TNF at weeks 0, 2, 6, 14, 30 and 46. Patients are represented as responders [closed circles] and non-responders [opened circles] at weeks 14 or 46. Plot lines show median ± range taking into account responders and non-responders. False discovery rate-corrected *p*-value: *< 0.05; **< 0.01; and ***< 0.001. [C] Expression of α4β7 determined by flow cytometry in blood memory CD4$^+$CD45RA$^-$ cells is shown at days 0, 1 and 4 after *in vitro* culture in complete media [CM] in the absence of vedolizumab. A representative sample is shown from a UC patient at week 46 after starting vedolizumab treatment. Cycloheximide [CHX] at 5 μg/mL was added under the indicated conditions. One representative experiment out of three is shown.

αE-expressing cells are abundant in the healthy intestinal lamina propria within both the intra-epithelial and the lamina propria compartments [Figure 6A]. In agreement with the literature,[24,33] the lymphocyte subset with the highest percentage of αEβ7$^+$ in the colon was CD8$^+$ T cells, followed by CD4$^+$ T cells [Figure 6B], while plasma cells did not express αEβ7 [data not shown]. It has been proposed that α4β7$^+$ T cells give rise to αEβ7$^+$ T cells.[34] To test this hypothesis, we measured the effect that blocking α4β7 would have on αEβ7 cells. Remarkably, treatment with vedolizumab, despite significantly reducing the number of α4β7$^+$ T cells in the mucosa, had no significant effect on the percentage of αEβ7$^+$ T lymphocytes [Figure 6C], suggesting that αEβ7 precursors may not be entering the mucosa primarily through α4β7.

Nonetheless, vedolizumab, but not anti-TNF, did significantly increase the number of circulating αEβ7 memory T cells as soon as 14 weeks after treatment initiation [Figure 6D]. This observation supports a role for α4β7 on αEβ7$^+$ T-cell recirculation.

Indeed, we looked into α4β7 expression on both CD8$^+$ and CD4$^+$ αEβ7$^+$ cells and found that while only a small percentage of αEβ7$^+$ cells in the colon expressed α4β7, over 50% of circulating αEβ7 cells co-expressed the homing receptor α4β7 [data not shown].

3.5 Vedolizumab's effects on intestinal α4β1$^+$ cellular migration

In addition to α4β7, leukocytes can employ α4β1 to migrate into the inflamed intestine by binding to vascular cell-adhesion molecule [VCAM]-1. In contrast to α4β7 [Figure 4A], the percentage of α4β1$^+$ CD4$^+$ T cells was significantly increased and to a similar extent in the mucosa of UC patients with active disease naïve to anti-TNF therapy or in those with prior anti-TNF failure [treated with vedolizumab] [week 0] [Figure 7]. Both treatments significantly reduced the percentage of α4β1$^+$ CD4$^+$ T cells, suggesting a non-specific effect on α4β1-expressing T-cell populations.

4. Discussion

Despite its approval in 2014 for the treatment of UC and Crohn's disease, the mechanisms of action underlying vedolizumab are still not fully understood. Vedolizumab was initially developed under the assumption that it would specifically block recruitment of mucosal homing T cells to the intestine,[10] thereby dampening inflammation in a gut-restricted manner. Data from primates revealed rapid amelioration of colitis in cotton-top tamarins treated with an anti-α4β7 mAb[19] [ACT-1, a murine IgG1 version of vedolizumab] and a significant reduction in β7$^+$ cells, as well as neutrophils and macrophages in the intestine of cynomolgus monkeys receiving vedolizumab.[9,26] Both studies also showed the saturation of α4β7 on blood lymphocytes by ACT-1 or vedolizumab. Subsequently, nearly full occupancy [>95% of α4β7 being occupied by vedolizumab] in peripheral[3,28] and intestinal cells[25] was reported in IBD patients. Our data confirm that vedolizumab administered at established clinical doses results in full α4β7 occupancy in blood T and B cells, as well as in intestinal lymphocytes, except for a proportion of patients in whom we detected unoccupied α4β7 on intestinal CD8$^+$ cells, despite the full saturation of CD4$^+$ and plasma cells in the same patient and tissue. A previous study had also shown incomplete occupancy of α4β7 in intestinal memory CD3$^+$ T cells.[25] This observation may reflect differential tissue antibody penetration depending on the cellular compartment [e.g. intra-epithelial compartment]. Importantly, incomplete CD8$^+$ T-cell receptor occupancy in tissue was not associated in our study with lack of therapeutic response during follow-up.

The number of lymphocytes populating the intestinal mucosa can change significantly, as we have confirmed here, in active UC due to increased cell recruitment and resident cell proliferation.[35–38] Consequently, any therapy that targets inflammation and/or immune responses [immunosuppressors, corticosteroids, anti-TNF, anti-leukocyte trafficking therapy, etc.] could potentially have an impact on the total numbers and proportions of cells populating the intestinal mucosa of patients. While total lymphocytes are increased in active UC, we show that the proportion of lymphocytes [20–30% of T cells and 80% of plasma cells] that express α4β7 was not significantly altered by inflammation. In contrast, there was an enrichment of α4β1-expressing CD4$^+$ T cells in UC patients. α4β7 and α4β1, which are not co-expressed in most lymphocytes[10,11] [and data not shown], mediate cell recruitment to the inflamed colonic mucosa by binding to independent receptors on the endothelial lining [MAdCAM-1 and VCAM-1, respectively] and therefore are independently regulated, as we confirm here.

The observation that both vedolizumab and anti-TNF decrease the proportion of α4β1$^+$ CD4$^+$ T cells in the mucosa suggests that this effect is secondary to the control of inflammation exerted by both antibodies, as neither of them directly targets α4β1. In contrast to the β1 integrin, we demonstrate that only vedolizumab, but not anti-TNF, specifically reduces the proportion of α4β7-expressing CD4$^+$ and CD8$^+$ T cells in the mucosa, while eventually increasing circulating memory CD4$^+$α4β7$^+$ T cells and CD19$^+$IgD$^-$α4β7$^+$ B cells. These findings support the initial hypothesis that vedolizumab acts by reducing α4β7-mediated T-cell homing to the gut[9,30] and are in agreement with recent data on mucosal CD4$^+$ cells.[39]

There is surprisingly little consensus on the effects of vedolizumab on resident tissue and tissue-infiltrating T and B cells, with no study thus far providing information beyond the 14-week induction period. Indeed, a recent article examined total CD3$^+$ cells [as well as B cells and plasma cells] in lamina propria and the intra-epithelial compartment, separately, in a mixed cohort of UC and Crohn's disease patients starting vedolizumab, and reported no change in the percentages at the end of induction[40]. In contrast, in an independent group of UC and Crohn's disease patients, the authors describe significant changes in total T-cell lamina propria populations after anti-TNF treatment. Inconsistencies in the total T-cell numbers, and therefore in the ability of a treatment to modify them significantly, can be explained by disease heterogeneity between cohorts, especially when including Crohn's disease patients, who are a highly heterogenous population. More surprising is their observation that anti-TNF treatment had no effect on innate-related signatures, which is a consistently reported result in the literature.[37,41–43] In addition, their study focused on total T cells and did not analyse the effects of treatment specifically on α4β7$^+$ cells, which our current results show is the cell population in which greater differences can be observed between vedolizumab and anti-TNF-treated patients. In contrast, our results show that both anti-TNF and vedolizumab significantly modulate the transcriptional signature related to innate and acquired immune responses in responding patients. In agreement with these effects, the overall number of intestinal lymphocytes is markedly reduced by treatment with either therapy.

Beyond promoting homing to the intestinal mucosa, α4β7 [but not α4β1] mediates the recruitment of naïve T and B cells to gut-associated secondary lymphoid tissues,[21,22] suggesting that vedolizumab could also impact primary responses to new antigens present in the intestinal lumen. As per the limitations of our

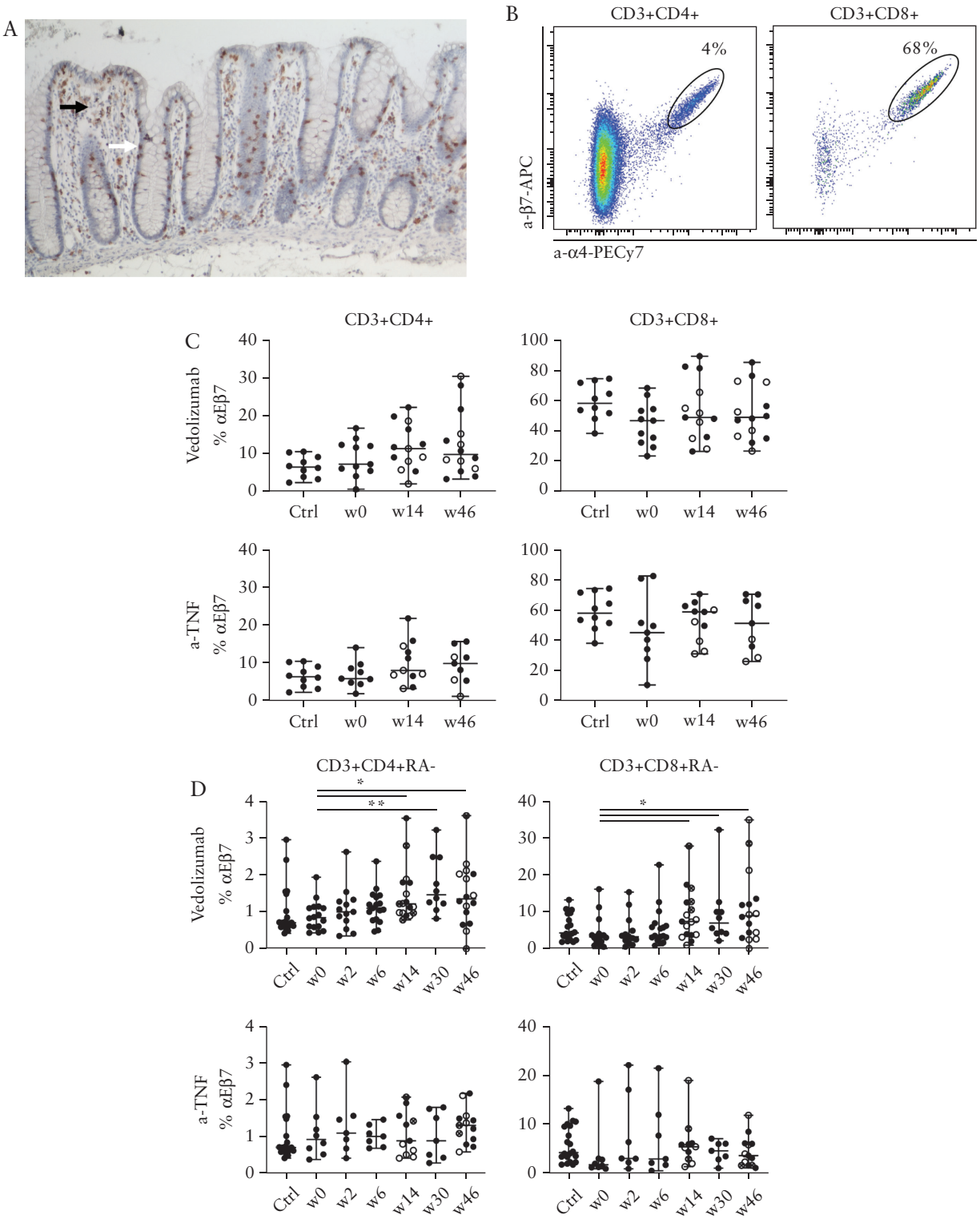

Figure 6. Differential effect of vedolizumab on intestinal or circulating αEβ7⁺ T cells. [A] Representative immunohistochemistry of a control colon stained with anti-αE antibody. Positive cells are abundant along the intestinal epithelium [white arrow] and the lamina propria [black arrow]. [B] Representative plots showing expression of αE and β7 on intestinal $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells. [C] Graphs showing the percentage of αEβ7⁺ in $CD3^+CD4^+$ and $CD3^+CD8^+$ intestinal lymphocytes determined by flow cytometry at weeks 0, 14 and 46. [D] Percentage of αEβ7⁺ within memory $CD3^+CD4^+$ and $CD3^+CD8^+$ peripheral blood circulating cells shown at weeks 0, 2, 6, 14, 30 and 46. All frequencies were measured in controls [Ctrl] and UC patients treated with vedolizumab or anti-TNF. Patients are represented as responders [closed circles] and non-responders [open circles] at weeks 14 or 46. Plot lines show median ± range taking into account responders and non-responders. False discovery rate-corrected *p*-value: *< 0.05; **< 0.01; and ***< 0.001.

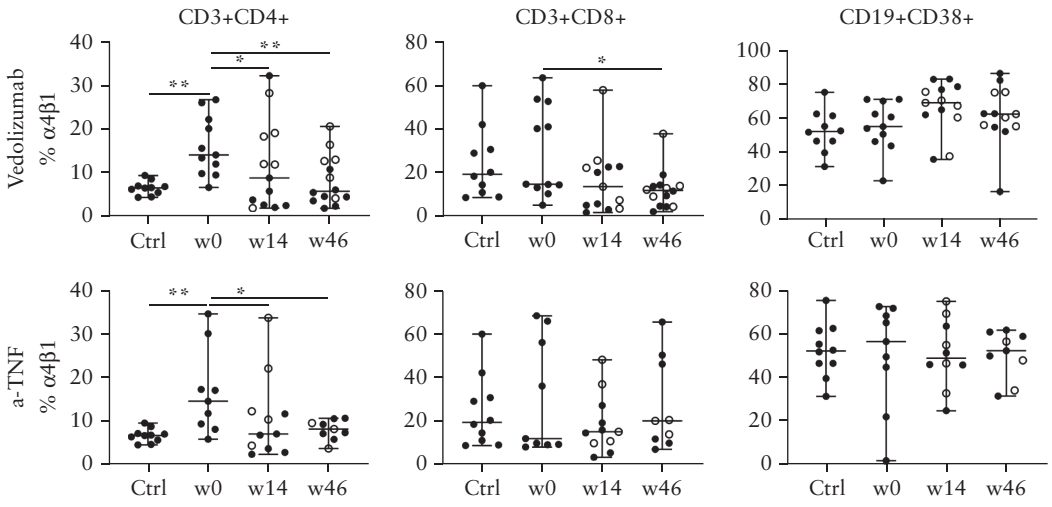

Figure 7. Vedolizumab and anti-TNF decrease the frequency of α4β1T cells but not plasma cells. Percentage of α4β1⁺ cells in CD3⁺CD4⁺, CD3⁺CD8⁺ and CD19⁺CD38⁺ intestinal lymphocytes determined by flow cytometry using co-staining with anti-α4 and anti-β1 antibodies. [A] UC patients receiving vedolizumab at weeks 0, 14 and 46. [B] UC patients receiving anti-TNF at weeks 0, 14 and 46. Patients are represented as responders [closed circles] and non-responders [open circles] at weeks 14 or 46. Plot lines show median ± range taking into account responders and non-responders. False discovery rate-corrected *p*-value: *< 0.05; **< 0.01; and ***< 0.001.

observational study, we did not have access to inductive sites [i.e. lymph nodes draining the intestinal mucosa or Peyer patches in the small intestine], and thus we could not directly assess the effects of vedolizumab on leukocyte circulation within these sites. Nonetheless, previous evidence supports the role of α4β7-mediated interactions in maintaining those lymphoid aggregates abundant in the ileal mucosa.[44] Uzzan and colleagues studied the ileum and left colon [both uninvolved mucosa] of UC patients with concomitant chronic HIV infection. In this unique cohort they showed that vedolizumab, even in the absence of inflammation, decreased the number of naïve T cells and B cells in lymphoid aggregates. In line with this, another study demonstrated the role that α4β7-dependent cell recruitment plays in mounting humoral immune responses to oral vaccines, which was markedly reduced in vedolizumab-treated patients, while still preserving the response to systemic vaccination.[45] Altogether, these observations would support a role for vedolizumab that goes beyond the canonical view of exclusively interfering with effector memory T-cell recruitment to the lamina propria. Nonetheless, whether it plays a role in controlling chronic intestinal inflammation remains to be proven.

Our results, in agreement with others,[44] also rule out a role for α4β7 on plasma cell recruitment to the intestinal mucosa. Indeed, while both vedolizumab and anti-TNF significantly reduced the overall plasma cell population in the colonic mucosa of UC patients, the remaining plasma cells showed high percentages of α4β7 expression.[44] This observation suggests that, in contrast to other lymphocytes, plasma cells may not use α4β7 as a receptor for intestinal homing. Beyond binding to endothelial adhesion molecules [MAdCAM-1 and VCAM-1], α4β7 has been described to use the extracellular matrix protein fibronectin as ligand.[46] The functional role of α4β7–fibronectin interactions, specifically on plasma cells, has not been yet elucidated but we hypothesize that it could drive intracellular signals required for cell survival, differentiation or activation similarly to the integrin–fibronectin interactions[47].

An intriguing observation that can be made from our study is vedolizumab's apparent lack of effect on intestinal αEβ7⁺ T cells. The role of αEβ7⁺ T cells in IBD remains ambiguous. While some studies suggest that αEβ7⁺ lymphocytes may promote inflammation in UC patients,[48] these cells abundantly populate the healthy intestinal mucosa and intra-epithelial compartment and recent studies have found no changes during inflammation of this subset.[33,49]

It has been assumed that αEβ7 may arise from α4β7 cells entering the gut and responding to environmental cues.[34] Nonetheless, the majority of αEβ7 cells found within,[50] or migrating towards,[51] the epithelial layer express low levels of α4β7. Here, we noted that the majority of αEβ7⁺ T cells in the colon did not co-express α4β7. Moreover, vedolizumab treatment, while significantly decreasing the number of α4β7 cells, had no clear impact on the intestinal αEβ7 compartment. This would suggest that αEβ7 can arise independently of gut homing α4β7⁺ lymphocytes, a hypothesis that would require further confirmation.

In striking contrast to the intestinal subset and as previously reported,[33] most circulating αEβ7 co-expressed α4β7 and their numbers increased in the blood of patients treated with vedolizumab. However, the significance of circulating double β7-expressing T cells remains an unanswered question. We propose that they may represent a population of gut-imprinted cells that circulate between inductive sites and blood with the ability to enter the mucosa in an α4β7-dependent manner. In addition, αEβ7⁺ in the blood were CD69⁻ [data not shown], supporting the view that they do not constitute tissue-resident T cells escaping mucosal tissues.[52] While vedolizumab blocks the homing of these cells, as supported by their accumulation in blood, our data also support the conclusion that α4β7-independent pathways are required to maintain the αEβ7 population in the gut. Indeed, to our knowledge no data support the likelihood that αEβ7 can only be acquired by α4β7⁺ cells; rather, all activated T cells can quickly upregulate αE and β7 in response to TGF-β, with a bias of αE transcription towards CD8⁺ cells.[53]

In summary, while vedolizumab can reduce the overall T- and plasma cell populations in the intestine, and downregulate the transcription of genes related to innate immune responses, we propose that these indirect effects are secondary to the control of inflammation. We base this conclusion on the fact that anti-TNF, which does not selectively block α4β7, results in similar decreases in T-cell and plasma cell infiltration, as well as transcriptional downregulation of immune-related genes. In contrast, vedolizumab [but not anti-TNF]

depletes α4β7⁺ CD4⁺ and CD8⁺ T cells in the colon, while preserving the proportion of α4β7⁺ plasma cells. Vedolizumab also appears to block the homing of circulating αEβ7⁺ T cells, although this does not result in a significant change in αEβ7⁺ within the mucosa, suggesting that other mechanisms beyond α4β7 are required to maintain that key pool of cells.

Finally, our study represents a first step towards understanding the specific and unique effects of anti-α4β7 mAbs compared to anti-TNF. We believe further studies will help us decipher key predictors of response within the identified direct mechanisms of action that, in a manner similar to predictors, should be unique to each specific drug type.[54]

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Conflicts of Interest

M.V., A.G.-T., A.M.C., M.C.M., H.B.-M., Mi.E., M.A., E.T. and A.F.-C. have no conflicts of interest to declare. Ma.E. has acted as a consultant, assessor or scientific advisory member for AbbVie, MSD, Tillotts Pharma, Pfizer and Takeda and received research grants for MSD and Abbvie. E.R. has served as a speaker, a consultant, or an advisory member for MSD, Abbvie, Takeda, Janssen, Pfizer, Ferring, Fresenius-Kabi and Adacyte. I.O. has received consulting fees from Abbvie and lecture fees from Abbvie, Takeda and Jansen. J.P. has received unrestricted institutional grants from Abbvie, MSD and Pfizer, and consultancy fees from AbbVie, Boehringer Ingelheim, Celgene, Genentech, GoodGut, GSK, Janssen, MSD, Nestle, Novartis, Oppilan, Pfizer, Progenity, Shire, Robarts, Roche, Takeda, Theravance and TiGenix. A.S. has served as a speaker or scientific advisor for Pfizer, Genentech, GSK, Boehringer Ingelheim and Roche, and has received grants from Roche, Genentech, Abbvie, Scipher Medicine, Robarts, Pfizer and GSK.

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Author Contributions

M.V. and A.S. designed the study, analysed and interpreted data and drafted and revised the manuscript. M.V., A.G.-T., Mi.E., H.B.-M. and M.A. performed experiments and analysed the data. J.P. and Ma.E. carefully reviewed the manuscript for intellectual content. I.O., A.F.-C., E.R., J.P. and Ma.E. recruited patients and performed endoscopic and clinical follow-up. E.T. and M.C.M. collected samples and provided technical support. A.M.C. analysed the data and performed statistical analysis. A.S. supervised the study and obtained financial support.

Supplementary Data

Supplementary data are available at *ECCO-JCC* online.

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