

Original Article

AlphaE Integrin Expression Is Increased in the Ileum Relative to the Colon and Unaffected by Inflammation

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Abstract

Background: Recent findings suggest that α E expression is enriched on effector T cells and that intestinal α E⁺T cells have increased expression of inflammatory cytokines. α E integrin expression is a potential predictive biomarker for response to etrolizumab, a monoclonal antibody against β 7 integrin that targets both α 4 β 7 and α E β 7. We evaluated the prevalence and localization of α E⁺ cells as well as total α E gene expression in healthy and inflammatory bowel disease patients.

Methods: α E⁺ cells were identified in ileal and colonic biopsies by immunohistochemistry and counted using an automated algorithm. Gene expression was assessed by quantitative reverse-transcriptase polymerase chain reaction.

Results: In both healthy and inflammatory bowel disease patients, significantly more α E⁺ cells were present in the epithelium and lamina propria of ileal compared with colonic biopsies. α E gene expression levels were also significantly higher in ileal compared with colonic biopsies. Paired biopsies from the same patient showed moderate correlation of α E expression between the ileum and colon. Inflammation did not affect α E expression, and neither endoscopy nor histology scores correlated with α E gene expression. α E expression was not different between patients based on concomitant medication use except 5-aminosalicylic acid.

Conclusion: α E⁺ cells, which have been shown to have inflammatory potential, are increased in the ileum in comparison with the colon in both Crohn's disease and ulcerative colitis, as well as in healthy subjects. In inflammatory bowel disease patients, α E levels are stable, regardless of inflammatory status or most concomitant medications, which could support its use as a biomarker for etrolizumab.

Key Words: Integrin alphaE; CD103; inflammatory bowel disease; ulcerative colitis; Crohn's disease.

1. Introduction

Inflammatory bowel disease [IBD] is characterized by intestinal inflammation that results from an abnormal immune response to the gut microbiome.¹ IBD encompasses both Crohn's disease [CD], characterized by transmural and patchy disease distribution that can occur anywhere along the gastrointestinal tract and most commonly affects the ileum or ileum and proximal colon, and ulcerative colitis [UC], which presents as continuous mucosal inflammation restricted to the colon and rectum. IBD pathobiology is probably driven by complex interactions between host genetics, immune dysregulation, the gastrointestinal microbiota and environmental factors that are incompletely understood.²

Interactions between integrins and adhesion molecules mediate homing of leukocytes to peripheral tissues.³ The binding of $\alpha 4\beta 7$ integrin to mucosal vascular addressin cell adhesion molecule 1 [MAdCAM-1], an adhesion molecule expressed on high endothelial venules in the lamina propria [LP] and Peyer's patches, is involved in gut-specific leukocyte trafficking. Clinical studies have demonstrated the importance of this pathway, as blocking antibodies against $\beta 7$ integrin,⁴ the $\alpha 4\beta 7$ integrin⁵⁻⁷ heterodimer or MAdCAM-1⁸ have all demonstrated clinical efficacy in UC. Etrolizumab is a humanized monoclonal anti- $\beta 7$ antibody with a dual mechanism of action, inhibiting both $\alpha 4\beta 7$:MAdCAM-1-mediated lymphocyte trafficking to the gut mucosa as well as $\alpha E\beta 7$:E-cadherin-mediated lymphocyte retention in epithelium. The αE integrin subunit [also known as CD103] exclusively pairs with the $\beta 7$ integrin subunit to form an $\alpha E\beta 7$ integrin heterodimer, which is expressed on a subset of intestinal T cells as well as dendritic cells [DCs], mast cells⁹ and innate lymphocytes.¹⁰ Because αE pairs only with $\beta 7$, detection of αE alone by gene or protein expression implies rather than directly demonstrates that $\alpha E\beta 7$ is expressed by a cell; hence, αE^+ cells are referred to as such throughout. αE^+ cells are retained in the gut epithelium through binding of $\alpha E\beta 7$ integrin on their cell surface to E-cadherin expressed on epithelial cells. Blockade of both $\alpha 4\beta 7$ and $\alpha E\beta 7$ resulted in decreased accumulation of adoptively transferred T cells in the intestinal mucosa in comparison with blockade of $\alpha 4\beta 7$ alone in a humanized mouse model.¹¹ In a phase 2 study of etrolizumab in UC patients, crypt-associated αE^+ cells were decreased following etrolizumab treatment. In a *post hoc* analysis in the same study, higher rates of remission were observed in etrolizumab-treated UC patients with above-median levels of αE in baseline colonic biopsies.⁴

Initial work in mouse models associated αE expression with regulatory T cell function.^{12,13} In humans, emerging data support an inflammatory rather than a regulatory role for αE^+ T cells. Th9 and Th17 effector T cells in the peripheral blood have increased expression of αE in comparison with regulatory T cells.¹¹ FOXP3⁺ cells in the gut mucosa rarely have co-expression of αE ,^{11,14} and sorted intestinal αE^+ T cells have low gene expression of FOXP3 and associated regulatory co-stimulatory molecules and cytokines.¹⁴ Instead, intestinal αE -expressing T cells have higher levels of pro-inflammatory cytokines and effector molecules compared with T cells lacking expression of αE .^{14,15} In addition, studies have shown that αE -expressing T cells can target and kill epithelial cells *in vitro* and may mediate localized tissue damage.¹⁶ Early studies suggest a higher prevalence of αE^+ cells in the ileum and proximal colon,¹⁷⁻²⁰ indicating that αE^+ cells may be of particular interest for CD pathobiology.

We undertook studies to evaluate αE^+ cells in UC, CD and healthy subjects, both to characterize the prevalence and localization in the intestine as well as to test the association of these cells with active inflammation. Our findings on the distribution and stability

of these measurements in IBD are relevant given the potential for biopsy-based predictive biomarkers for etrolizumab.

2. Methods

2.1. Patients

Three cohorts of patients were included in this study. In the first cohort, immunohistochemistry [IHC] was used to perform a retrospective analysis on formalin-fixed biopsy samples taken from IBD and healthy control patients undergoing endoscopic assessment as part of standard clinical care in Newcastle upon Tyne, UK [CD patients, $n = 48$; UC patients, $n = 72$; healthy control subjects, $n = 91$]. In this cohort [IHC cohort], active disease in the ileum or colon was defined as visible mucosal inflammation endoscopically and histological confirmation of active inflammation via examination by a pathologist. Inactive disease in the ileum or colon was defined as lack of visible inflammation at endoscopy and histological inflammation by pathologist examination.

A second cohort [qPCR cohort] comprised IBD patients and healthy control subjects enrolled into a prospective single-time point observational study²¹ designated as EMBARK. All patients underwent a full ileo-colonoscopy, and biopsies were taken in both the ileum and the colon. A subset of patients had mucosal biopsy-derived RNA available for analysis [CD patients, $n = 63$; UC patients, $n = 30$; healthy subjects, $n = 14$].²² Active disease in EMBARK was defined as a Mayo Clinic endoscopic subscore [MCSe] ≥ 2 for UC patients and as a Simple Endoscopic Score for Crohn's Disease [SES-CD]²³ ≥ 7 , or ≥ 4 for ileum-only CD patients.²⁴ Patients with endoscopic evidence of inflammation whose biopsies lacked active histological inflammation were excluded from the analysis.

Biopsy variability was evaluated in a third cohort consisting of UC patients [$n = 6$] and healthy subjects [$n = 5$] enrolled into an observational study with no therapeutic intervention. Four biopsies were collected from each subject at 2-cm intervals along the sigmoid colon. Disease activity was evaluated by MCS endoscopic subscore and histological assessment.

Written informed consent was obtained in accordance with research and ethics committee [REC] [Newcastle and North Tyneside 1 REC 10/H0906/41 & Newcastle and North Tyneside 2 REC 22/02] and institutional review boards' [IRB] approval at each study site. These studies were performed according to the principles of the *Declaration of Helsinki*.

2.2. Immunohistochemistry

Biopsies were fixed in formalin and processed for immunohistochemistry as previously described.⁴ Briefly, 4- μ m sections were stained using a Benchmark XT [Ventana Medical Systems] autostainer with anti-integrin αE antibody [EPR4166; Abcam]. Images were acquired using the Olympus Nanozoomer automated slide scanning platform [Hamamatsu Photonics] and analysed in the MATLAB software package [version R2012a; The MathWorks] to generate total αE^+ cell counts. Individual cells were identified using an algorithm based on radial symmetry,²⁵ and cells were scored as positive or negative for 3,3'-diaminobenzidine [DAB] staining using a blue-normalized algorithm to identify brown pixels.²⁶ Epithelium was identified as previously described^{4,14} and αE^+ cells within one cell diameter of the epithelium were identified and counted. LP areas were identified using a specific combination of morphology, cell density and manual annotation that included only LP and excluded crypts, surface epithelium and lymphoid aggregates.

2.3. Gene expression analysis

Biopsies were collected and processed as previously described.²² Briefly, intestinal biopsies were collected from uninfamed or inflamed areas [if present] as judged by the endoscopist. RNA was isolated using the RNeasy kit [Qiagen], and samples with high RNA quality [RNA integrity number ≥ 5] were included in the analysis [$n = 229$]. Quantitative PCR [qPCR] was performed using the BioMark HD System [Fluidigm] using primer sets specific for *CD3 ϵ* , *ITGAE* [αE] or *TMEM55B* [Applied Biosystems]. Technical replicates with a standard deviation ≥ 2 or a standard deviation ≥ 1 with an average Ct < 25 were removed from the analysis. Gene expression was normalized to *TMEM55B* using the ΔC_t method. Paired analysis of ileal and colonic biopsies only included uninfamed biopsies from the same patient. Paired analysis of uninfamed–inflamed biopsies was performed on biopsies from the same anatomical region within each patient.

2.4. Statistical analysis

Statistical analysis was performed using Prism5 [GraphPad software] and JMP11 [SAS]. Patient demographics were assessed using the chi-squared test. Statistical significance between independent groups was assessed using the Mann–Whitney test. Paired analysis was performed using the Wilcoxon matched-pairs signed-rank test. Correlation was assessed by Spearman's rho. Mean and standard deviation are shown on all plots. For concordance analysis, αE status was defined as being above the median in both organs or below the median in both organs. For variability analysis, the standard deviation was calculated for the biopsies from a single patient [inpatient] and for the biopsies from all patients at a particular collection distance [interpatient]. A binomial test was performed to assess the differences between inpatient and interpatient variability. Statistical significance [$P < 0.05$] was defined based on nominal p values, and different levels were noted on graphs as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

3. Results

Patient demographics between cohorts were generally similar, with some key differences in disease activity and duration as well as current therapies [Table 1]. There were more active CD patients in the IHC cohort than in the qPCR cohort [active CD: IHC cohort, 63%, vs qPCR cohort, 22%; $P < 0.0001$]. UC patients in the IHC cohort were older than in the qPCR cohort [median age: IHC cohort, 54 years, vs qPCR cohort, 41 years; $P < 0.01$]. A longer disease duration was reported in the CD qPCR cohort in comparison with the IHC cohort [qPCR cohort, median disease duration of 10 years, vs IHC cohort, median disease duration of 3 years; $P < 0.05$]. Therapeutic use was variable across the UC cohorts, with corticosteroid use more common in the UC qPCR cohort in comparison with the IHC and biopsy variability cohorts [qPCR cohort, 50%, vs IHC cohort, 24%, vs biopsy variability cohort, 33%; $P < 0.05$], while anti-tumour necrosis factor [anti-TNF] use was more frequent in the UC biopsy variability cohort in comparison with the qPCR cohort [qPCR cohort, 7%, vs biopsy variability cohort, 50%; $P < 0.0001$]. Finally, more UC patients were on 5-aminosalicylic acid [5-ASA] therapy in the IHC cohort in comparison with the other two cohorts [IHC cohort, 67%; qPCR cohort, 7%; biopsy variability cohort, 50%; $P < 0.0001$].

Biopsies from both the terminal ileum and the colon contained readily detectable αE^+ cells in both the LP and the epithelium [Figure 1a]. For this analysis, an average of αE^+ cells across

the region was generated for patients with multiple biopsies. Quantification of total αE^+ cells by IHC showed that the proportion of αE^+ cells was significantly higher in the ileum in comparison with the colon of patients with UC [ileum, 1334 ± 726.7 cells/mm², vs colon, 575.0 ± 292.6 cells/mm²; $P < 0.001$] and CD [ileum, 891.6 ± 442.1 cells/mm², vs colon, 357.9 ± 153.0 cells/mm²; $P < 0.0001$] as well as control subjects [ileum, 1107.0 ± 518.7 cells/mm², vs colon, 478.4 ± 279.5 cells/mm²; $P < 0.0001$] [Figure 1b]. The ileum had similar numbers of αE^+ cells in IBD patients and healthy subjects. In contrast, there were significant differences in cell density between patient groups and healthy controls in the colon. Of the three cohorts, CD patients had reduced numbers of αE^+ cells in the colon [CD 357.9 ± 153 cells/mm²] in comparison with healthy controls [478.4 ± 279.5 cells/mm², $P < 0.05$] and UC patients [575 ± 292.6 cells/mm², $P < 0.0001$]. The increased density of αE^+ cells in colons of patients with UC was also significantly higher than in those of healthy subjects [$P < 0.05$] [Figure 1b].

E-cadherin, the ligand for $\alpha E\beta 7$, is expressed on epithelial cells in the gut and probably functions to retain αE^+ cells at the mucosal barrier. The density of αE^+ cells in the epithelium was consistently higher in the ileum compared with the colon in patients with CD [ileum, 2678 ± 1601 cells/mm², vs colon, 1392 ± 652 cells/mm²; $P < 0.01$], those with UC [ileum, 4097 ± 2767 cells/mm², vs colon, 2217 ± 1024 cells/mm²; $P < 0.01$] and healthy controls [ileum, 3910 ± 2003 cells/mm², vs colon, 1869 ± 1062 cells/mm²; $P < 0.0001$] [Figure 1c]. Although the majority of αE^+ cells were localized in the epithelium,^{9,14} αE^+ cells were also observed to localize to the LP with similar trends to intra-epithelial αE^+ cells. LP αE^+ cells were increased in the ileum compared with the colon in CD patients [ileum, 861 ± 430.5 cells/mm², vs colon, 342.6 ± 148.2 cells/mm²; $P < 0.0001$], UC patients [ileum, 1300 ± 717.6 cells/mm², vs colon, 551.6 ± 286.6 ; $P < 0.001$] and healthy subjects [ileum, 1060 ± 498 cells/mm², vs colon, 459 ± 270.2 cells/mm²; $P < 0.0001$] [Supplementary Figure 1].

Gene expression analysis in biopsies supported the IHC-based findings of increased αE^+ cells in the ileum compared with the colon. For this analysis, a single biopsy was used for each anatomical region per patient; uninfamed biopsies, when available, were used to represent each patient. A two- to four-fold increase in *ITGAE* [αE] gene expression was observed in ileal biopsies in comparison with colonic biopsies across both IBD [CD ileum, -1.18 ± 0.90 , vs CD colon, -2.50 ± 1.11 ; $P < 0.0001$; UC ileum, -1.38 ± 0.44 , vs UC colon, -2.84 ± 0.92 ; $P < 0.0001$] and control samples [ileum, -1.08 ± 1.06 , vs colon, -2.71 ± 0.85 ; $P < 0.001$] [Figure 2a]. An overall increase in *CD3 ϵ* gene expression in ileal biopsies compared with colonic biopsies was also observed in patients with CD [ileum, 0.001 ± 0.99 , vs colon, -1.19 ± 1.12 ; $P < 0.0001$] and control samples [ileum, 0.71 ± 0.58 , vs colon, -0.97 ± 0.90 ; $P < 0.001$] [Figure 2b]. No significant differences in αE and *CD3 ϵ* gene expression were observed in inpatient paired uninfamed–inflamed ileal or colonic biopsies [Figure 2c and d].

The proportion of αE^+ cells in the ileum and colon were correlated in paired patient biopsies, with both total αE^+ cells and epithelial αE^+ cells demonstrating a correlation between the two anatomical regions [$\rho = 0.48$, $P < 0.0001$; and $\rho = 0.39$, $P < 0.01$, respectively] [Figure 3a and b]. A significant correlation of αE normalized to *CD3 ϵ* gene expression levels was observed in patient-paired ileal and colonic biopsies [$\rho = 0.36$, $P < 0.01$] [Figure 3c]. αE gene expression alone was not correlated [data not shown]. Biopsies used for this analysis were almost exclusively paired uninfamed samples, as paired inflamed ileal–colonic biopsies were not available in the majority of patients.

Table 1. Patient demographics

		Number of males, n [%]	Age, median [range], years	Disease dur- ation, me- dian [range], years	Smoking, n [current/former/never/unknown]	Immuno- modulators, n [%]	Anti-TNF, n [%]	Corticosteroids, n [%]	5-ASA, n [%]
IHC Cohort	Healthy [n = 91]	40 [44.0%]	45 [20-78]	-	NA	-	-	-	-
	CD [n = 48]***	18 [37.5%]	41.5 [18-82]	3 [0-43]*	16/10/20/2	14 [29.2%]	5 [10.4%]	10 [20.8%]	8 [16.7%]
	active [n = 30]	14 [46.7%]	41.5 [18-75]	2 [0-36]	9/7/13/1	9 [30.0%]	4 [13.3%]	8 [26.7%]	5 [16.7%]
	inactive [n = 18]	4 [22.2%]	41 [23-82]	9 [0-43]	7/3/7/1	5 [27.8%]	1 [5.6%]	2 [11.1%]	3 [16.7%]
	UC [n = 72]	42 [58.3%]	54 [21-83]**	6.5 [0-53]	4/26/32/10	12 [16.7%]	0 [0.0%]	17 [23.6%]	48 [66.7%]****
	active [n = 35]	23 [65.7%]	51 [21-83]	1 [0-53]	1/13/18/3	7 [20.0%]	0 [0.0%]	9 [25.7%]	16 [45.7%]
	inactive [n = 37]	19 [51.4%]	54 [29-80]	14 [0-46]	3/13/14/7	5 [13.5%]	0 [0.0%]	8 [21.6%]	32 [86.5%]
	Healthy [n = 14]	4 [28.6%]	53 [26-61]	-	2/5/7/0	-	-	-	-
	CD [n = 63]	31 [49.2%]	35 [18-65]	10 [0-45]	17/15/31/0	21 [33.3%]	4 [6.3%]	20 [31.7%]	20 [31.7%]
	active [n = 14]	7 [50.0%]	35 [19-59]	6.5 [2-45]	5/4/5/0	2 [14.3%]	0 [0.0%]	4 [28.6%]	7 [50.0%]
inactive [n = 49]	24 [49.0%]	37 [18-65]	10 [0-33]	12/11/26/0	19 [38.8%]	4 [8.2%]	16 [32.7%]	13 [26.5%]	
UC [n = 30]	14 [46.7%]	40.5 [22-64]	7.5 [1-39]	5/9/16/0	7 [23.3%]	2 [6.7%]	15 [50.0%]*	2 [6.7%]	
active [n = 13]	6 [46.2%]	38 [24-62]	7 [1-19]	2/4/7/0	3 [23.1%]	1 [7.7%]	7 [53.8%]	1 [7.7%]	
inactive [n = 17]	8 [47.1%]	48 [22-64]	8 [1-39]	3/5/9/0	4 [23.5%]	1 [5.9%]	8 [47.1%]	1 [5.9%]	
Healthy [n = 5]	3 [60%]	34 [26-46]	-	0/2/3/0	-	-	-	-	
Biopsy Variability Cohort	UC [n = 6]	4 [66.7%]	35.5 [25-62]	5.5 [0-10]	1/2/3/0	2 [33.3%]	3 [50.0%]****	2 [33.3%]	3 [50.0%]
	active [n = 3]	3 [100%]	38 [28-62]	3 [3-9]	0/2/1/0	1 [33.3%]	2 [66.7%]	2 [66.7%]	1 [33.3%]
	inactive [n = 3]	1 [33.3%]	33 [25-50]	8 [0-10]	1/0/2/0	1 [33.3%]	1 [33.3%]	0 [0.0%]	2 [66.7%]

5-ASA, 5-aminosalicylic acid; CD, Crohn's disease; IHC, immunohistochemistry; NA, not available; qPCR, quantitative polymerase chain reaction; TNF, tumour necrosis factor; UC, ulcerative colitis.

Active disease was defined by endoscopic disease activity confirmed by pathologist examination [see Methods]. Statistically significant differences are noted and further discussed in the text, * $P < 0.05$, ** $P < 0.01$,

**** $P < 0.0001$, using the Mann-Whitney test.

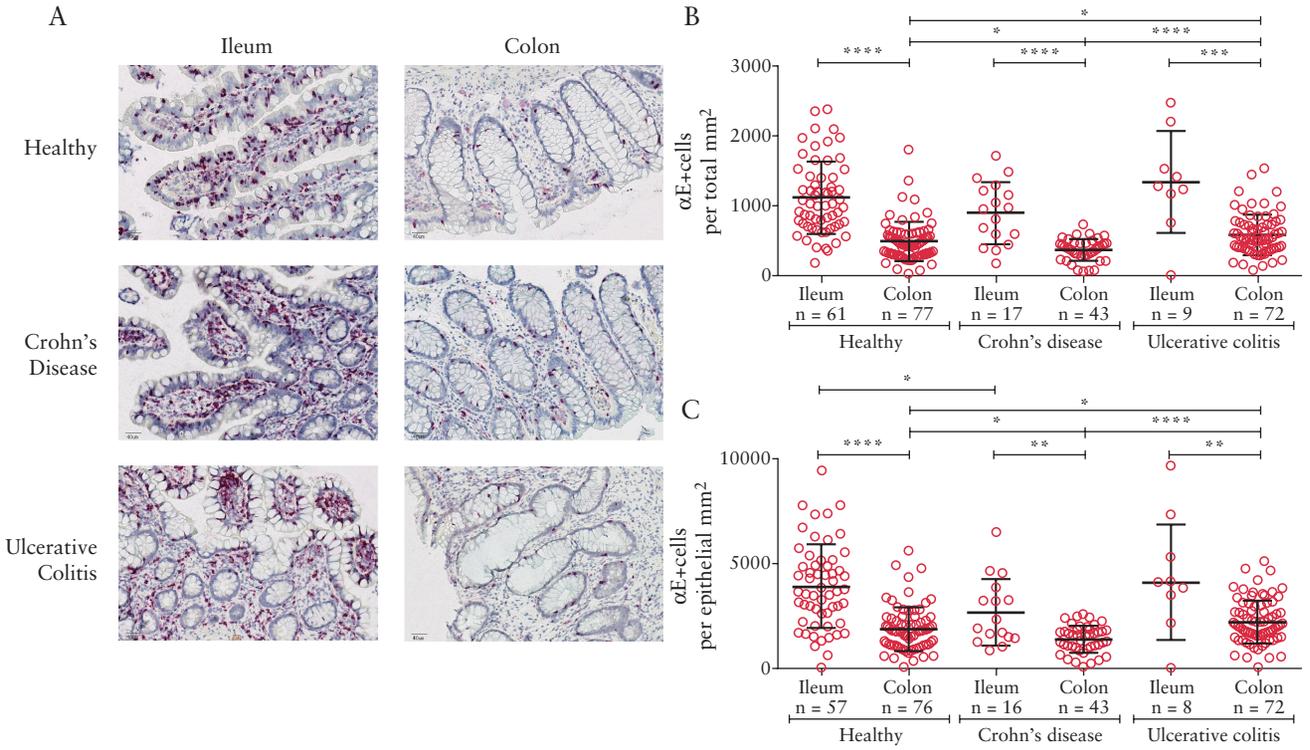


Figure 1. αE^+ cells are more frequent in the ileum in comparison with the colon. [a] Representative images of αE staining [red] in ileal and colonic biopsies. Images shown are from samples with the median staining density by patient group and anatomical location. [b] All αE^+ cells and [c] αE^+ cells associated with epithelial crypts were counted in biopsies from healthy controls and patients with UC or CD. Lines and error bars show the mean \pm SD. CD, Crohn's disease; SD, standard deviation; UC, ulcerative colitis.

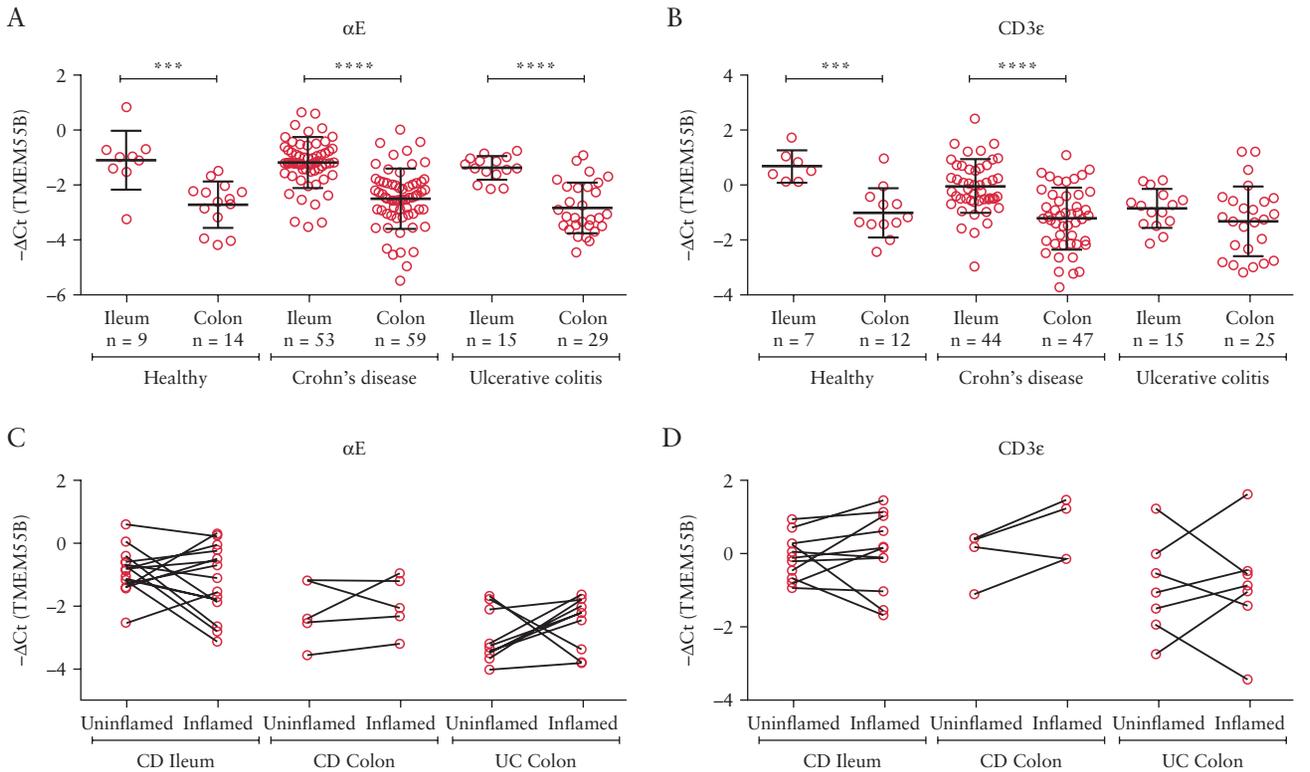


Figure 2. ITGAE [αE] and CD3 ϵ gene expression is higher in the ileum than colon. [a–b] αE and CD3 ϵ gene expression were measured in ileal and colonic biopsies from healthy control subjects and IBD patients. Gene expression was normalized to TMEM55B. Lines and error bars show the mean \pm SD. [c–d] Paired biopsies were taken from inflamed and uninfamed areas of the ileum and colon of IBD patients and evaluated for αE and CD3 ϵ gene expression relative to TMEM55B. CD, Crohn's disease; IBD, inflammatory bowel disease; SD, standard deviation; UC, ulcerative colitis.

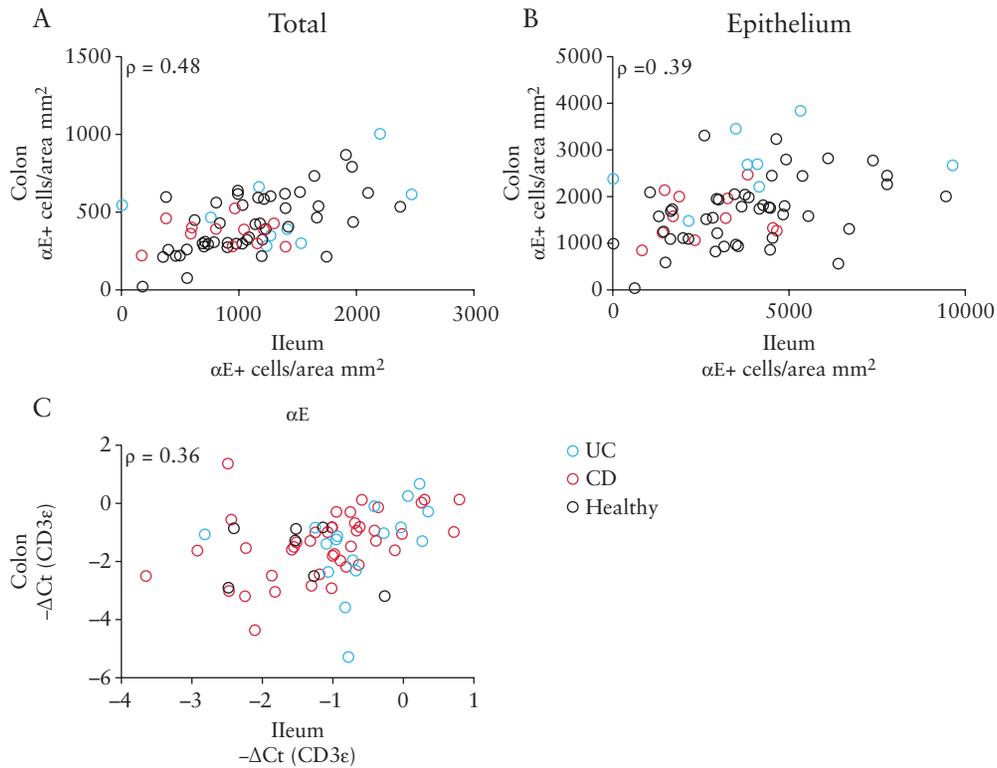


Figure 3. αE levels are correlated in paired ileal and colonic biopsies from the same subject. Paired ileal and colonic biopsies were evaluated in healthy subjects and IBD patients for correlation of [a] total αE^+ cells or [b] epithelial-associated αE^+ cells. [c] αE gene expression normalized to CD3 ϵ expression was also evaluated for correlation between paired ileal and colonic biopsies. CD, Crohn's disease; IBD, inflammatory bowel disease; UC, ulcerative colitis.

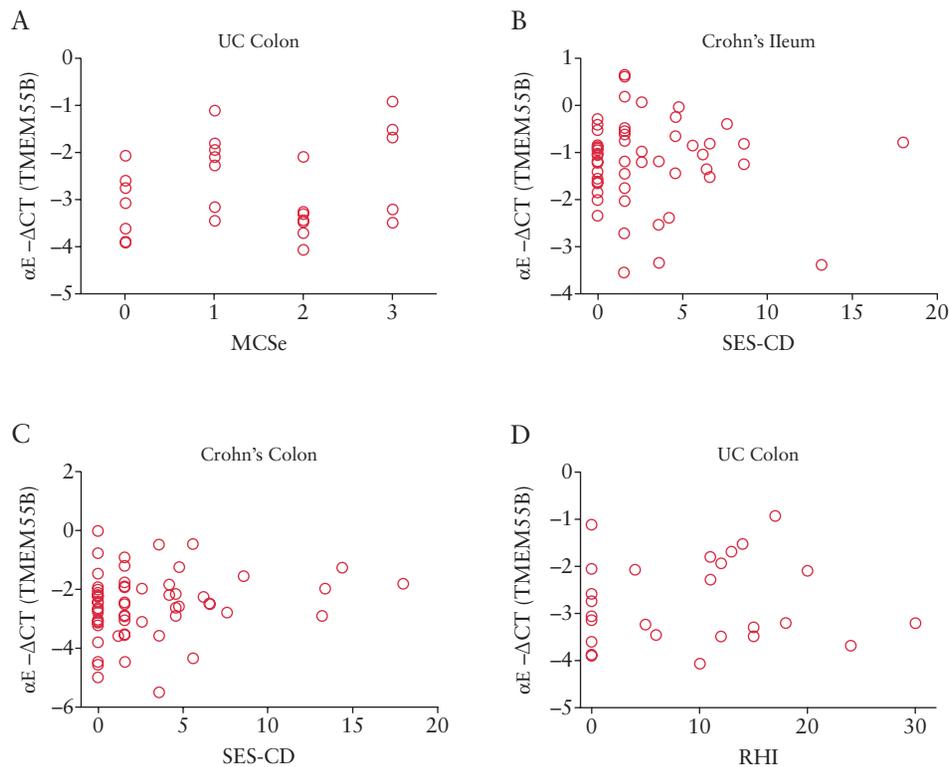


Figure 4. Endoscopic and histological inflammation are not correlated with αE levels. Endoscopic activity scores were not correlated with αE gene expression in [a] UC patients or CD patients with active [b] ileal or [c] colonic disease. [d] Histological disease activity as measured by the Roberts Histology Index [RHI] was also not correlated with αE gene expression in UC patients. All gene expression levels are normalized to TMEM55B. CD, Crohn's disease; MCSe, Mayo Clinic endoscopic subscore; SES-CD, Simple Endoscopic Score for Crohn's Disease; UC, ulcerative colitis.

α E high and low status by either IHC or qPCR was examined for concordance between the colon and ileum using a median cutoff. By IHC, 50% of CD patients were concordant for α E status in the colon and ileum, while 66% of healthy subjects and 56% of UC patients were similarly concordant for α E status. Using α E gene expression normalized to CD3e, 74% of CD patients, 43% of healthy subjects and 57% of UC patients were concordant for α E status by qPCR in the colon and ileum.

Disease activity was assessed by both endoscopy and histology and correlated with biopsy α E gene expression from the same anatomical region. Uninflamed biopsies were used for this analysis when available. No correlation was observed between α E gene expression and endoscopy score in UC or CD [Figure 4a–c]. There was a similar lack of correlation between α E gene expression and histological inflammation in UC as assessed by the Robarts Histology Index [RHI]²⁷ [Figure 4d]. No difference was observed for α E⁺ cell density or α E gene expression based on inflammation or disease activity status [Supplementary Figure 2a and b].

To evaluate α E gene expression variability over a defined segment of the colon, a series of biopsies were taken 2 cm apart in the sigmoid colon of healthy and UC subjects. Differences between inpatient and interpatient variability were evaluated. First, we took the biopsy location with the lowest standard deviation between the patients of each subset [healthy SD = 0.446, UC SD = 0.589]. We then used a binomial test to assess if the inpatient variability was significantly less than the lowest interpatient variability. We found that inpatient variability was significantly lower in healthy patients [$P < 0.05$] but not in UC patients. There were no significant differences in variability and α E gene expression levels between healthy subjects and UC patients [Figure 5a and b].

The effect of concomitant medication use, including corticosteroids and immunosuppressants, on α E⁺ cell density and α E gene expression was evaluated in both the IHC and qPCR cohorts. No significant differences were observed between patients based on current use of corticosteroids, immunosuppressants or biological therapy. There was a small, but significant [$P < 0.05$], increase in CD colonic α E gene expression with 5-ASA use [Supplementary Figures 4–7]. No difference in α E⁺ cells was observed in patients treated with 5-ASA in the IHC cohort.

4. Discussion

The pathobiology of IBD is probably driven by complex interactions between the gut microbiota and the immune system, with other

factors such as epithelial barrier function and environmental cues playing key roles. Therapeutics that target inflammatory cytokines, such as tumour necrosis factor alpha [TNF- α] and interleukin [IL]-12/IL-23,²⁸ as well as gut-selective leukocyte trafficking,²⁹ are approved for IBD. Despite the widespread use of anti-TNF- α therapies, fewer than half of CD^{30,31} and UC patients^{32,33} treated with these agents achieve remission. Furthermore, vedolizumab, which targets the α 4 β 7 integrin, is less efficacious in CD than UC and among patients who have previously failed anti-TNF- α therapies.^{6,7,34,35} The diversity of factors driving IBD pathobiology and the significant proportion of patients who fail to respond to biological therapy suggests that patient heterogeneity is a key driver for response to therapy in IBD.³⁶ Biomarkers that enable identification of the underlying pathobiology of disease would enable more precise treatment regimens and increase the likelihood of clinical benefit for individual patients. For a tissue-based biomarker, stable expression, irrespective of concomitant medications, site of biopsy and degree of inflammation, along with reasonable cost and ease of use, is desirable.

To better understand α E as a predictive biomarker of clinical remission in response to etrolizumab in IBD patients, we sought to further characterize α E gene expression and α E⁺ cell density in the gut in IBD patients and healthy control subjects. In this study, we found that both α E⁺ cell numbers and α E gene expression are two- to four-fold higher in the ileum in comparison with the colon in normal and IBD biopsies. Higher levels of pro-inflammatory cytokines have been observed in intestinal α E-expressing T cells compared with T cells lacking expression of α E in both healthy and UC colon.¹⁴ In our study, a small but significant reduction in α E⁺ cells was observed in colonic CD in comparison with healthy or UC colon; this reduction was not found in α E levels in the qPCR cohort. Further analysis showed no correlation with disease duration or number of medications [data not shown]. A previous study using flow cytometry and a mixture of ileal and colonic biopsies also found a reduced number of α E⁺ cells in IBD biopsies.³⁷ The effect on α E⁺ cell numbers and inflammatory cytokine production by these cells should be further investigated in CD, as cell numbers may not wholly reflect inflammatory potential. Correlation between α E⁺ cells was observed between matched ileal and colonic biopsies. α E gene expression normalized to CD3e, a marker gene for T cells, was also correlated between these two anatomical regions. Across our cohorts, approximately two-thirds of patients were concordant [α E high/ α E high or α E low/ α E low] for α E status in the colon and in the ileum when a median cutoff was used for patient categorization.⁴ Differences in α E levels were not impacted by inflammation status of the biopsy or

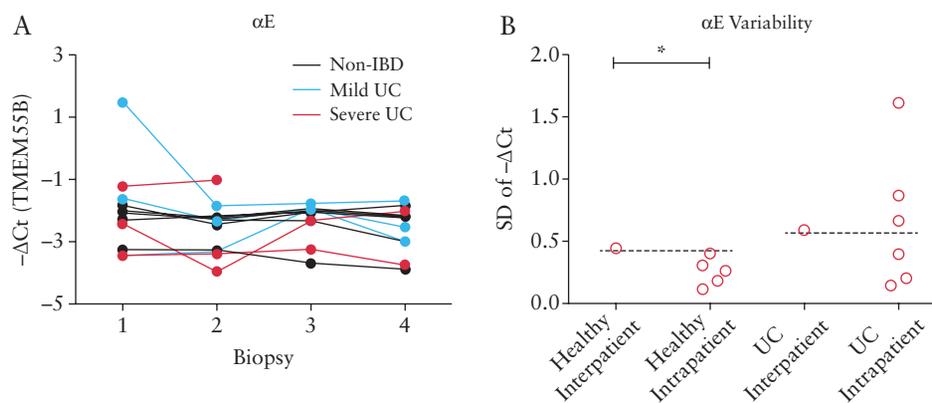


Figure 5. Intrapatient variability of α E gene expression levels is lower than interpatient variability. [a] Serial colonic biopsies were taken from healthy subjects and UC patients and evaluated for α E gene expression. [b] α E gene expression variability by disease indication. All gene expression levels are normalized to TMEM55B. Lines and error bars show the mean \pm SD. IBD, inflammatory bowel disease; SD, standard deviation; UC, ulcerative colitis.

concomitant medications, except for a small effect of 5-ASA that is not currently understood. Greater variability was observed between patients than within patients when α E gene expression was assessed across multiple biopsies from the sigmoid colon, suggesting that α E could serve as a biomarker to stratify patients, with low inpatient sampling variability. Caveats to this study include the retrospective nature of the analysis and the small sample size, particularly for the inpatient variability analysis.

As dictated by the clinical standard of care, IBD patients routinely undergo endoscopy and/or flexible sigmoidoscopy with concurrent mucosal biopsies to document the severity of histological disease activity and to screen for dysplasia. Despite the ready availability of mucosal biopsies from patients with IBD, biopsy-based predictive biomarker approaches to identifying patients likely to respond to targeted therapies have not yet been used for IBD. The identification and validation of such a predictive biomarker requires careful characterization of many possible sources of variability of biomarker expression including inflammation status and the intrinsic anatomical variability in biomarker levels, either within or between intestinal segments.

The effect of inflammation on α E expression was not tested in the etrolizumab phase 2 UC study, as all patients enrolled in the study had evidence of active endoscopic disease [endoscopic subscore ≥ 2], and biopsies were taken from an inflamed region in a standardized area of the sigmoid colon without paired sampling of uninfamed mucosa. A significant decrease in the proportion of crypt-associated α E⁺ cells and α E gene expression was observed following etrolizumab treatment.⁴ In our study, we observed no effect of inflammation status on α E levels, either through paired biopsies taken in areas judged by the endoscopist to be inflamed and uninfamed, or by correlation with endoscopy or histology score.

The differences in α E levels between different anatomical regions may provide an additional challenge when areas of active inflammation are irregularly distributed throughout the intestine, such as in CD. Here, inflamed regions, in particular the ileum, may be beyond the reach of the endoscope because of technical challenges including inflammatory or fibrotic stricturing. Previous work has described increased α E levels in the proximal colon in comparison with the distal colon.^{17,19,20} We found that α E expression was increased in the ileum in comparison with the sigmoid colon. Furthermore, α E expression in the colon and ileum were moderately correlated; this finding could be useful in patients in whom biopsy of the ileum is not possible. For this study, we used median cutoffs based on the sample population to designate high or low α E status; further work will be required to prospectively evaluate cutoffs for their potential predictive value.

Variability of gene expression or α E⁺ cell numbers within an anatomical intestinal segment may also affect the interpretation of a biopsy-based diagnostic. Previous work on inflammatory biomarkers has shown that 1–6 biopsies may be required to reduce sampling error to less than 25%.³⁸ Because α E expression does not appear to be influenced by inflammation, its variability would be expected to be less than that of an inflammatory gene such as TNF- α . We evaluated a small cohort of UC patients and healthy controls to determine whether interpatient variability was greater than inpatient variability for α E gene expression and found less variability within a given patient than between patients. This finding indicates that sampling error differences may be small enough to support a biopsy-based biomarker test. Because α E expression does not appear to be influenced by inflammation, its variability would be expected to be less than that of an inflammatory gene such as TNF- α or IP-10.³⁸

The stability of α E expression makes it favourable for biomarker development.

A key role for α 4 β 1-mediated trafficking to the ileum was observed in an adoptive transfer model using effector T cells from CD patients,³⁹ suggesting that the blockade of α 4 β 7 alone may not be sufficient to induce remission in all CD patients. UC patients with higher α E expression in baseline endoscopic mucosal biopsies as determined by qPCR or IHC had an enrichment of remission following etrolizumab treatment in an exploratory *post hoc* analysis of the phase 2 clinical study.^{4,15} These data suggest that the pathobiology of disease in patients with increased α E expression in gut tissue may be preferentially targeted by etrolizumab treatment.¹⁵ In this study, we confirm^{17,18} and extend the finding of a higher prevalence of α E⁺ cells in the ileum and proximal colon, a site of inflammation for a substantial proportion of CD patients. The additional blockade of α E β 7-mediated interactions may provide an additional mechanism of action in CD.

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Conflict of Interest

R.I., J.E.-A., A.S. and M.E.K. are all employees of Genentech, a member of the Roche Group. Financial or non-financial research support, educational funds or compensation for consulting, speaking or teaching has been received from: J.M.: AbbVie, Ferring, Genentech, Takeda, Tillotts; J.K.: Genentech, GlaxoSmithKline, Intercept Pharmaceuticals; C.L.: Genentech, Takeda. AL and MB have nothing to disclose.

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Author Contributions

J.C.M., J.A.K., C.A.L. and M.E.K. participated in the design of the study, W.A.F., L.R., M.R.B., A.K.L., J.C.M. and C.A.L. participated in patient accrual and data collection, R.I., J.E.A., A.S., C.A.L. and M.E.K. performed data analysis. All authors were members of the writing group and participated in development of the report, agreed on the content, reviewed drafts and approved the final version.

Supplementary Data

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

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