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

Ryan Ichikawa, a, * Christopher A. Lamb, b, c, *
Jeff Eastham-Anderson, a, Alexis Scherl, a, Laura Raffals, d
William A. Faubion, d, Miriam R. Bennett, b, Anna K. Long, e
John C. Mansfield, b, c, John A. Kirby, b, Mary E. Keir a

a Genentech Research and Early Development, South San Francisco, California, USA
b Newcastle University, Newcastle upon Tyne, UK
Department of Gastroenterology, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK
c Department of Gastroenterology and Hepatology, Mayo Clinic, Rochester, Minnesota, USA
d Division of Gastroenterology and Hepatology, Mayo Clinic, Rochester, Minnesota, USA
e Department of Cellular Pathology, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK

Corresponding author: Mary E. Keir, PhD, Genentech Research and Early Development, 1 DNA Way, Mail stop 231c, South San Francisco, CA 94080, USA. Tel: (650) 467-6852; Fax: (650) 742-4863; Email: keir.mary@gene.com

*These authors contributed equally to the manuscript.

Abstract

Background: Recent findings suggest that αE expression is enriched on effector T cells and that intestinal αE+ T cells have increased expression of inflammatory cytokines. αE integrin expression is a potential predictive biomarker for response to etrolizumab, a monoclonal antibody against β7 integrin that targets both α4β7 and αEβ7. We evaluated the prevalence and localization of αE+ cells as well as total αE gene expression in healthy and inflammatory bowel disease patients.

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

Conclusion: αE+ 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, αE 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. 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.

Interactions between integrins and adhesion molecules mediate homing of leukocytes to peripheral tissues. The binding of α4β7 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 β7 integrin, the α4β7 heterodimer or MAdCAM-1 have all demonstrated clinical efficacy in UC. Etrolizumab is a humanized monoclonal anti-β7 antibody with a dual mechanism of action, inhibiting both α4β7 and E-cadherin-mediated lymphocyte trafficking to the gut mucosa as well as αEβ7:E-cadherin-mediated lymphocyte retention in epithelium. The αEβ7 integrin subunit [also known as CD103] is expressed specifically by the β7 integrin subunit to form an αEβ7 integrin heterodimer, which is expressed on a subset of intestinal T cells as well as dendritic cells [DCs], mast cells and innate lymphocytes. Because αEβ7 pairs only with β7, detection of αE alone by gene or protein expression implies rather than directly demonstrates that αEβ7 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 αEβ7 integrin on their cell surface to E-cadherin expressed on epithelial cells. Blockade of both α4β7 and αEβ7 resulted in decreased accumulation of adoptively transferred T cells in the intestinal mucosa in comparison with blockade of α4β7 alone in a humanized mouse model. 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.

Initial work in mouse models associated αE expression with regulatory T cell function. 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. FOXP3+ cells in the gut mucosa rarely have co-expression of αE, and sorted intestinal αE+ T cells have low gene expression of FOXP3 and associated regulatory co-stimulatory molecules and cytokines. Instead, intestinal αE-expressing T cells have higher levels of pro-inflammatory cytokines and effector molecules compared with T cells lacking expression of αE. In addition, studies have shown that αE-expressing T cells can target and kill epithelial cells in vitro and may mediate localized tissue damage. Early studies suggest a higher prevalence of αE+ cells in the ileum and proximal colon, 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 study 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]. 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] ≥ 7, or ≥ 4 for ileum-only CD patients. 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. Briefly, 4-μm 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, and cells were scored as positive or negative for 3,3′-diaminobenzidine [DAB] staining using a blue-normalized algorithm to identify brown pixels. Epithelium was identified as previously described 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. Briefly, intestinal biopsies were collected from uninflamed 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 CD3e, ITGAE [αE] or TMEM53B [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 TMEM53B using the ΔCt method. Paired analysis of ileal–colonic biopsies only included uninflamed biopsies from the same patient. Paired analysis of uninflamed–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 [intrapatient] and for the biopsies from all patients at a particular collection distance [interpatient]. A binomial test was performed to assess the differences between intrapatient 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 colonos of patients with UC was also significantly higher than in those of healthy subjects [P < 0.05] [Figure 1b].

E-cadherin, the ligand for αEβ7, is expressed on epithelial cells within 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, 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; uninflamed 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 CD3e 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 CD3e gene expression were observed in intrapatient paired uninflamed–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 [r = 0.48, P < 0.001; and r = 0.39, P < 0.01, respectively] [Figure 3a and b]. A significant correlation of αE normalized to CD3e gene expression levels was observed in patient-paired ileal and colonic biopsies [r = 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 uninflamed samples, as paired inflamed ileal-colonic biopsies were not available in the majority of patients.
| IHC Cohort      | Healthy [n = 91] | 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%] |
|-----------------|------------------|------------|---------------|-----------|-------------|------------|----------|-----------|----------|
|                  | CD [n = 48]***  | 11 [23.5%] | 41.5 [21–83]** | 6.5 [1–39] | 4/26/32/10 | 12 [16.7%] | 0 [0.0%] | 17 [23.6%] | 48 [66.7%]**** |
|                  | active [n = 30] | 11 [36.7%] | 41.5 [21–75] | 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] | 3 [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 [1–39] | 4/26/32/10 | 12 [16.7%] | 0 [0.0%] | 17 [23.6%] | 48 [66.7%]**** |
|                  | active [n = 35] | 23 [65.7%] | 51 [21–75] | 1 [0–33] | 1/13/18/3 | 27 [77.1%] | 0 [0.0%] | 9 [25.7%] | 16 [45.7%] |
|                  | inactive [n = 37] | 19 [51.4%] | 54 [29–80] | 10 [0–46] | 5/13/14/7 | 5 [13.5%] | 0 [0.0%] | 8 [21.6%] | 32 [86.5%] |

| qPCR Cohort19    | 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%] |

| Biopsy Variability Cohort | Healthy [n = 5] | 3 [60%] | 34 [26–46] | - | 0/2/3/0 | - | - | - | - |
| 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.
**AlphaE Integrin Expression in IBD**

**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 ± SD. CD, Crohn’s disease; SD, standard deviation; UC, ulcerative colitis.

**Figure 2.** ITGAE [αE] and CD3ε gene expression is higher in the ileum than colon. (a–b) αE and CD3ε 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 ± SD. (c–d) Paired biopsies were taken from inflamed and uninflamed areas of the ileum and colon of IBD patients and evaluated for αE and CD3ε 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 CD3ε 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 Robarts 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.
αE 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 αE status in the colon and ileum, while 66% of healthy subjects and 56% of UC patients were similarly concordant for αE status. Using αE gene expression normalized to CD3ε, 74% of CD patients, 43% of healthy subjects and 57% of UC patients were concordant for αE status by qPCR in the colon and ileum.

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

To evaluate αE 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 intrapatient 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 intrapatient variability was significantly less than the lowest interpatient variability. We found that intrapatient variability was significantly lower in healthy patients [P < 0.05] but not in UC patients. There were no significant differences in variability and αE gene expression levels between healthy subjects and UC patients [Figure 5a and b].

The effect of concomitant medication use, including corticosteroids and immunosuppressants, on αE+ cell density and αE 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 αE gene expression with 5-ASA use [Supplementary Figures 4–7]. No difference in αE+ 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 CD30,31 and UC patients32,33 treated with these agents achieve remission. Furthermore, vedolizumab, which targets the α4β7 integrin, is less efficacious in CD than UC and among patients who have previously failed anti–TNF-α therapies.4,26 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.34 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 αE as a predictive biomarker of clinical remission in response to etrolizumab in IBD patients, we sought to further characterize αE gene expression and αE+ cell density in the gut in IBD patients and healthy control subjects. In this study, we found that both αE+ cell numbers and αE 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 αE-expressing T cells compared with T cells lacking expression of αE in both healthy and UC colon.35 In our study, a small but significant reduction in αE+ cells was observed in colonic CD in comparison with healthy or UC colon; this reduction was not found in αE 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 αE+ cells in IBD biopsies.36 The effect on αE+ 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 αE+ cells was observed between matched ileal and colonic biopsies. αE gene expression normalized to CD3ε, a marker gene for T cells, was also correlated between these two anatomical regions. Across our cohorts, approximately two-thirds of patients were concordant [αE high/αE high or αE low/αE low] for αE status in the colon and in the ileum when a median cutoff was used for patient categorization.4 Differences in αE levels were not impacted by inflammation status of the biopsy or

Figure 5. Intrapatient variability of αE gene expression levels is lower than interpatient variability. [a] Serial colonic biopsies were taken from healthy subjects and UC patients and evaluated for αE gene expression. [b] αE gene expression variability by disease indication. All gene expression levels are normalized to TMEM55B. Lines and error bars show the mean ± 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 αE gene expression was assessed across multiple biopsies from the sigmoid colon, suggesting that αE could serve as a biomarker to stratify patients, with low intrapatient sampling variability. Caveats to this study include the retrospective nature of the analysis and the small sample size, particularly for the intrapatient 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 αE 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 αE+ cells and αE gene expression was observed following etrolizumab treatment.4 In our study, we observed no effect of inflammation status on αE 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 αE 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 strictureing. Previous work has described increased αE levels in the proximal colon in comparison with the distal colon.31,32,33 We found that αE expression was increased in the ileum in comparison with the sigmoid colon. Furthermore, αE 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 αE status; further work will be required to prospectively evaluate cutoffs for their potential predictive value.

Variability of gene expression or αE+ 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%.34 Because αE 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-10.34 The stability of αE expression makes it favourable for biomarker development.

A key role for α4β7-mediated trafficking to the ileum was observed in an adoptive transfer model using effector T cells from CD patients,35 suggesting that the blockade of α4β7 alone may not be sufficient to induce remission in all CD patients. UC patients with higher αE 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.36 These data suggest that the pathobiology of disease in patients with increased αE expression in gut tissue may be preferentially targeted by etrolizumab treatment.37 In this study, we confirm37,38 and extend the finding of a higher prevalence of αE+ cells in the ileum and proximal colon, a site of inflammation for a substantial proportion of CD patients. The additional blockade of αEβ7-mediated interactions may provide an additional mechanism of action in CD.

Funding
This work was supported by F. Hoffmann-La Roche Ltd and by the Wellcome Trust [grant number 093885/Z/10/Z to C.A.L.]. C.A.L. is a Clinical Lecturer supported by the National Institute for Health Research [NIHR].

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.

Acknowledgments
This work was supported by the MRC/ESPRC Newcastle Molecular Pathology Node and by the Newcastle Academic Health Partners Bioresource. Wei Tew and May Witke provided support for sample processing and sample handling, respectively. The authors thank Oleg Mayba for advice on statistical analyses. ApotheCom provided editorial support for the preparation and submission of the manuscript.

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.

References
1. Marsal J, Agace WW. Targeting T-cell migration in inflammatory bowel disease. J Intern Med 2012;272:411–29.
2. Kaiser A, Zeissig S, Blumberg RS. Inflammatory bowel disease. Annu Rev Immunol 2010;28:573–621.
3. Hynes RO. Integrins: bidirectional, allosteric signaling machines. Cell 2002;110:673–87.
4. Vermeire S, O’Byrne S, Keir M, et al. Etrolizumab as induction therapy for ulcerative colitis: a randomised, controlled, phase 2 trial. Lancet 2014;384:309–18.

5. Sands BE, Feagan BG, Rutgeerts P, et al. Effects of vedolizumab induction therapy for patients with Crohn’s disease in whom tumor necrosis factor antagonist treatment failed. Gastroenterology 2014;147:618–27.e3.

6. Feagan BG, Rutgeerts P, Sands BE, et al.; GEMINI 1 Study Group. Vedolizumab as induction and maintenance therapy for ulcerative colitis. N Engl J Med 2013;369:699–710.

7. Sandborn WJ, Feagan BG, Rutgeerts P, et al.; GEMINI 2 Study Group. Vedolizumab as induction and maintenance therapy for Crohn’s disease. N Engl J Med 2013;369:711–21.

8. Reinsch W, Sandborn W, Danese S, et al. 901 A randomized, multicenter double-blind, placebo-controlled study of the safety and efficacy of anti-MAdCAM antibody PF-00547659 (PF) in patients with moderate to severe ulcerative colitis: results of the TURANDOT study. Gastroenterology 2015;148:S-1193.

9. Gorfu G, Rivera-Nieves J, Ley K. Role of beta7 integrins in intestinal lymphocyte homing and retention. Curr Mol Med 2009;9:836–50.

10. Fuchs A, Vermi W, Lee JS, et al. Intrathelial type 1 innate lymphoid cells are a unique subset of IL-12- and IL-15-responsive IFN-γ-producing cells. Immunity 2013;38:789–81.

11. Zundler S, Schillinger D, Fischer A, et al. Blockade of αEβ7 integrin suppresses accumulation of CD8+ and Th9 lymphocytes from patients with IBD in the inflamed gut in vivo. Gut 2017;66:1936–48.

12. Lehmann J, Huehn J, de la Rosa M, et al. Expression of the integrin alpha Ebeta 7 identifies unique subsets of CD25+ as well as CD25- regulatory T cells. Proc Natl Acad Sci U S A 2002;99:13031–6.

13. Banz A, Peixoto A, Pontoues C, Cordier C, Rocha B, Papiernik M. A unique subpopulation of CD4+ regulatory T cells controls wasting disease, IL-10 secretion and T cell homeostasis. Eur J Immunol 2003;33:2419–28.

14. Lamb CA, Mansfield JC, Tew GW, et al. αEβ7 integrin identifies subsets of pro-inflammatory colonic CD4+ T lymphocytes in ulcerative colitis. J Crohns Colitis 2017;11:610–20.

15. Tew GW, Hackney JA, Gibbons D, et al. Association between response to etrolizumab and expression of Integrin αE and Granzyme A in colon biopsies of patients with ulcerative colitis. Gastroenterology 2016;150:477–87.e9.

16. Roberts AI, O’Connell SM, Biancone L, Brolin RE, Ebert EC. Spontaneous cytotoxicity of intestinal intraepithelial lymphocytes: clues to the mechanism. Clin Exp Immunol 1993;94:327–32.

17. Kirby JA, Bone M, Robertson H, Hudson M, Jones DE. The number of intraepithelial T cells decreases from ascending colon to rectum. J Clin Pathol 2003;56:158.

18. Oshutani N, Watanabe K, Maeda K, et al. Differential expression of homing receptor CD103 on lamina propria lymphocytes and association of CD103 with epithelial adhesion molecules in inflammatory bowel disease. Int J Mol Med 2003;12:715–9.

19. Elewaut D, Van Damme N, De Keyser F, et al. Altered expression of alpha E beta 7 integrin on intraepithelial and lamina propria lymphocytes in patients with Crohn’s disease. Acta Gastroenterol Belg 1998;61:288–94.

20. Vezsey RS, Rosenzweig M, Shvetz DE, et al. Characterization of gut-associated lymphoid tissue (GALT) of normal rhesus macaques. Clin Immunol Immunopathol 1997;82:330–42.

21. Faubion WA Jr, Fletcher JG, O’Byrne S, et al. EMerging BiomARKers in Inflammatory Bowel Disease (EMARKAR) study identifies fecal calprotectin, serum MMP9, and serum IL-22 as a novel combination of biomarkers for Crohn’s disease activity: role of cross-sectional imaging. Am J Gastroenterol 2013;108:1891–900.

22. Colombel JF, Keir ME, Scherl A, et al. Discrepancies between patient-reported outcomes, and endoscopic and histological appearance in UC. Gut 2017;66:2063–8.

23. Daperno M, D’Haens G, Van Assche G, et al. Development and validation of a new, simplified endoscopic activity score for Crohn’s disease: the SES-CD. Gastroenterology Endosc 2004;60:505–12.

24. Feagan BG, Sands BE, Rossiter G, et al. Effects of Mongersen (GED-0301) on endoscopic and clinical outcomes in patients with active Crohn’s disease. Gastroenterology 2018;154:61–4.e6.

25. Vera M, van Diest PJ, Kornegoor R, Huismans A, Vergever MA, Pluim JP. Automatic nuclei segmentation in H&E stained breast cancer histopathology images. PLoS One 2013;8:e70221.

26. Brey EM, Lalani Z, Johnston C, et al. Automated selection of DAB-labeled tissue for immunohistochemical quantification. J Histochem Cytochem 2003;51:575–84.

27. Mosli MH, Feagan BG, Zou G, et al. Development and validation of a histological index for UC. Gut 2017;66:50–8.

28. Feagan BG, Sandborn WJ, Gasink C, et al.; UNITI-IM-UNITI Study Group. Ustekinumab as induction and maintenance therapy for Crohn’s disease. N Engl J Med 2016;375:1946–60.

29. Sandborn WJ, Colombel JF, Enns R, et al.; International Efficacy of Natalizumab as Active Crohn’s Therapy (ENACT-1) Trial Group; Evaluation of Natalizumab as Continuous Therapy (ENACT-2) Trial Group. Natalizumab induction and maintenance therapy for Crohn’s disease. N Engl J Med 2005;353:1912–25.

30. Hanauer SB, Sandborn WJ, Rutgeerts P, et al. Human anti-tumor necrosis factor monoclonal antibody (adalimumab) in Crohn’s disease: the CLASSIC-I trial. Gastroenterology 2006;130:322–33; quiz 591.

31. Targan SR, Hanauer SB, van Deventer SJ, et al. A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor alpha for Crohn’s disease. Crohn’s disease cA2 study group. N Engl J Med 1997;337:1029–35.

32. Rutgeerts P, Sandborn WJ, Feagan BG, et al. Infliximab for induction and maintenance therapy for ulcerative colitis. N Engl J Med 2005;353:2462–76.

33. Sandborn WJ, van Assche G, Reichnish W, et al. Adalimumab induces and maintains clinical remission in patients with moderate-to-severe ulcerative colitis. Gastroenterology 2012;142:57–65.e1–3.

34. Sands BE, Sandborn WJ, Van Assche G, et al. Vedolizumab as induction and maintenance therapy for Crohn’s disease in patients naïve to or who have failed tumor necrosis factor antagonist therapy. Inflamm Bowel Dis 2017;23:97–106.

35. Dulai PS, Singh S, Jiang X, et al. The real-world effectiveness and safety of vedolizumab for moderate-severe Crohn’s disease: results from the US VICTORY Consortium. Am J Gastroenterol 2016;111:1447–55.

36. Arron JR, Townsend MJ, Keir ME, Yaspan BL, Chan AC. Stratified medicine in inflammatory disorders: from theory to practice. Clin Immunol 2015;161:11–22.

37. Horjus Talabur Horje CS, Middendorp S, von Koolwijk E, Veta M, van Diest PJ, Kornegoor R, Huisman A, Viergever MA, Pluim JP. Automated selection of DAB-labeled tissue for immunohistochemical quantification. J Histochem Cytochem 2003;51:575–84.

38. Mosh MH, Feagan BG, Zou G, et al. Development and validation of a histological index for UC. Gut 2017;66:50–8.