


# Responsiveness to Vedolizumab Therapy in Ulcerative Colitis is Associated With Alterations in Immune Cell-Cell Communications

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**Background:** Ulcerative colitis (UC) and Crohn's disease are 2 types of inflammatory bowel disease (IBD), a group of chronic digestive disorders caused by aberrant immune responses to intestinal microbes. Although changes in the composition of immune cell subsets in the context of IBD have been previously described, the interactions and communication among cells are less well understood. Moreover, the precise mechanisms of action underlying many biologic therapies, including the anti- $\alpha 4\beta 7$  integrin antagonist vedolizumab, remain incompletely understood. Our study aimed to explore possible additional mechanisms through which vedolizumab acts.

**Methods:** We performed cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq) on peripheral blood and colon immune cells derived from patients with ulcerative colitis treated with the anti- $\alpha 4\beta 7$  integrin antagonist vedolizumab. We applied a previously published computational approach, NicheNet, to predict immune cell-cell interactions, revealing putative ligand-receptor pairs and key transcriptional changes downstream of these cell-cell communications (CCC).

**Results:** We observed decreased proportions of T helper 17 ( $T_H17$ ) cells in UC patients who responded to vedolizumab and therefore focused the study on identifying cell-cell communications and signals of  $T_H17$  cells with other immune cells. For example, we observed that colon  $T_H17$  cells from vedolizumab nonresponders were predicted to have a greater degree of interactions with classical monocytes compared with responders, whereas colon  $T_H17$  cells from vedolizumab responders exhibited more interactions with myeloid dendritic cells compared with nonresponders.

**Conclusions:** Overall, our results indicate that efforts to elucidate cell-cell communications among immune and nonimmune cell types may increase the mechanistic understanding of current and investigational therapies for IBD.

## Lay Summary

Compared to ulcerative patients unresponsive to vedolizumab, immune cell networks of ulcerative colitis patients responsive to vedolizumab have decreased proportion of  $T_H17$  and less pro-inflammatory signaling in the gut. Decreased pro- $T_H17$  and interleukin (IL)-1 signaling from classical monocytes and innate immunocytes may mediate this phenotype.

**Key Words:** single-cell RNA-sequencing, cell-cell communications, T cells, ulcerative colitis, vedolizumab

## Introduction

Ulcerative colitis (UC) is a type of inflammatory bowel disease (IBD) characterized by chronic intestinal inflammation that is limited to the mucosal layer of the colon. By contrast, Crohn's disease, another subtype of IBD, is characterized by transmural inflammation that can affect any part of the alimentary tract. Inflammatory bowel disease is thought to arise from a dysregulation of innate and adaptive immune responses to gut microbiota, but the pathophysiology is still not completely understood.<sup>1</sup>

Many types of immune and nonimmune cells have been implicated in the development of IBD, including CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes,<sup>2-4</sup> stromal cells,<sup>5</sup> macrophages,<sup>6</sup> innate

lymphoid cells,<sup>7</sup> and epithelial cells.<sup>8</sup> Many groups have sought to better characterize changes in the number, composition, and phenotype of immune cell changes within the peripheral blood and intestine in IBD patients.<sup>2-5,8,9</sup> However, the cell-cell interactions and signaling events that drive observed alterations of specific cell types are less well understood. Moreover, the precise mechanism of action of effective therapies for IBD, including the  $\alpha 4\beta 7$  integrin antagonist vedolizumab, remains poorly understood.

Vedolizumab is a humanized monoclonal antibody targeting the  $\alpha 4\beta 7$  integrin heterodimer expressed on the surface of lymphocytes that mediates migration into intestinal Peyer's patches and lamina propria via interaction with the

**Key Messages****What is already known?**

Vedolizumab, a monoclonal antibody that binds to  $\alpha 4\beta 7$  integrin, is thought to work primarily by blocking leukocyte trafficking into the gut; however, changes in innate immunity are consistently reported following treatment.

**What is new here?**

Inferred cell-cell communications show that signals from innate immune cells that promote  $T_H 17$  differentiation are decreased in vedolizumab responders.

**How can this study help patient care?**

This study provides evidence of a possible mechanism of action for vedolizumab that may decrease innate cell signaling promoting  $T_H 17$  cell differentiation, which may contribute to its clinical efficacy.

mucosal vascular adhesion molecule-1.<sup>10</sup> In vitro studies of vedolizumab show that the humanized antibody results in internalization of  $\alpha 4\beta 7$  integrin in lymphocytes without subsequent lymphocyte activation and does not activate antibody-dependent cellular cytotoxicity or complement-dependent cytotoxicity.<sup>11</sup> Vedolizumab's clinical potency has been presumed to be due to exclusion of gut-homing  $\alpha 4\beta 7$  integrin-expressing lymphocytes from intestinal tissue; its effect on innate immune cells is becoming increasingly appreciated. Recent studies reveal additional functions for  $\alpha 4\beta 7$  integrin signaling, including promoting T-cell proliferation and upregulating T helper 17 ( $T_H 17$ ) cell cytokine production and gene expression.<sup>12</sup> Single-cell analyses of gut tissues from IBD patients receiving vedolizumab have suggested significant interactions between  $T_H 17$  cells and inflammatory monocytes/macrophages.<sup>4,13,14</sup> These findings, combined with the observation that the innate immune response is also affected by  $\alpha 4\beta 7$  blockade, reinforce the notion that vedolizumab may exert actions beyond simply inhibiting lymphocyte trafficking.

Here, we used cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq),<sup>15</sup> which integrates transcriptional and proteomic profiling in single-cells, to analyze peripheral blood and colon immune cells from UC patients following treatment with vedolizumab, some of whom responded to therapy and others who did not. We annotated the cells using a published RNA-seq reference data set<sup>16</sup> and applied Differential NicheNet, an algorithm that infers ligand-receptor relationships from differential gene expression,<sup>17</sup> in order to identify putative cell-cell communications (CCCs) and predict secondary downstream transcriptional changes. We identified decreased proportions of colonic  $T_H 17$  cells in UC patients who responded to vedolizumab compared with those who failed to respond. We therefore focused the study on characterizing cell-cell communications of  $T_H 17$  cells with other immune cells. For example, we observed that colon  $T_H 17$  cells from vedolizumab nonresponders were predicted to have a greater degree of interactions with classical monocytes compared with responders, whereas colon  $T_H 17$  cells from vedolizumab responders exhibited more interactions with myeloid dendritic cells. Overall, our results indicate that efforts

to elucidate cell-cell communications among immune and nonimmune cell types may advance the mechanistic understanding of current and investigational therapies for IBD.

**Materials and Methods****Patient Criteria and Ethics**

This study was reviewed and approved by the Human Research Protections Program at the University of California, San Diego and the San Diego VA Healthcare. Patients with a diagnosis of ulcerative colitis and active endoscopic disease on minimal or no medical therapy were consented into the study; patient demographics and individual disease characteristics and prior treatment regimens are provided in [Supplementary Tables 1 and 2](#). Intestinal biopsies and peripheral blood were obtained from patients undergoing routine colonoscopy for clinical management of their disease.

**Human Peripheral Blood Mononuclear and Intestinal Cell Isolation**

Blood was collected directly into BD Vacutainer CPT cell preparation tubes (BD Biosciences) for isolation of CD45<sup>+</sup> immune cells, as previously described.<sup>18</sup> Prior to single-cell sequencing, frozen cells were thawed, washed, and passed through 70- $\mu$ m filters prior to antibody labeling and fluorescence-activated cell sorting (FACS) described below. Intestinal biopsies were obtained with endoscopic biopsy forceps from the rectum and collected in conical tubes with Hank's buffered saline solution (HBSS, Corning); they were then transferred into freezing buffer (10% [v/v] dimethyl sulfoxide [DMSO], 40% [v/v] complete Roswell Park Memorial Institute media [RPMI], 50% [v/v] fetal bovine serum [FBS]) and stored at  $-80^{\circ}\text{C}$ , as previously described.<sup>18</sup>

**Purification of CD45<sup>+</sup> Immune Cells and 10x Genomics Library Preparation and Sequencing**

Single-cell suspensions of peripheral blood mononuclear cell (PBMC) and intestinal cell preparations were incubated with a Fixable Viability Dye eFluor 780 (eBioscience, Invitrogen) on ice for 10 minutes and then incubated with BioLegend Human TruStain FcX Block for 10 minutes to block non-specific binding of the Fc receptor. An antibody mixture containing antihuman CD45 (2D1; BioLegend) and a 58-plex TotalSeq antibody cocktail generated using 1  $\mu$ g of each TotalSeq antibody was prepared in Cell Staining Buffer (BioLegend). Samples were incubated for 60 minutes on ice, then washed, and resuspended in complete RPMI. The CD45<sup>+</sup> immune cells were sorted on a FACS Aria2 (BD Biosciences) and collected in fresh complete RPMI. The FACS-purified CD45<sup>+</sup> immune cells were then washed and resuspended in PBS + 0.04% (w/v) bovine serum albumin (UltraPure BSA, Thermo Fisher Scientific). Single-cell libraries were prepared according to the protocol for 10x Genomics for Single Cell 3' Gene Expression v3.1, as previously described.<sup>18</sup> Single-cell RNA and protein libraries were pooled at equimolar amounts for a final 10 nM concentration and sequenced on a NovaSeq 6000 S4 Flow Cell (Illumina), as previously described.<sup>18</sup>

**Computational Analyses**

Single-cell analyses and computational analyses using Seurat and NicheNet were performed as previously published.<sup>18</sup> Raw sequencing data were fed through Cell Ranger pipeline (10x Genomics), and downstream processing was done

using Seurat v4.1.1.<sup>19</sup> The data were filtered to remove low quality cells and doublets with the following parameters: <200 features, >10% mitochondrial features, and >2500 features. Protein and RNA data sets were normalized, and then immune cell types were assigned labels using SingleR v.1.4.1<sup>20</sup> and manually verified with canonical protein markers according to [Supplementary Table 3](#). NicheNet, a cell-cell communications workflow, compares differentially expressed (DE) genes in designated receiver cells to the DE genes of possible sender cells to infer key ligand-receptor pairs between the senders and receivers. We used Differential NicheNet, the most updated version of NicheNet, to rank upstream ligands and receptors in senders and receivers that had at least 25 identified cells within their respective tissue and expressed by at least 10% of the cell population, as previously described.<sup>18</sup>

### Pathway Enrichment Analyses

The top 50 prioritized ligand-receptor pairs were inputs for ligand-receptor pathway analysis. For ligand-target pathway analysis, the ligand-targets were required to share the same highly ranked ligand-receptors upstream of targets. The top 2 ligand-sender pairs with the highest ligand target weights served as pathway analysis inputs.

### Statistical Analysis

GraphPad Prism 9 (GraphPad Software) was used for statistical analysis of cell type proportions and for subsequent graphical representations of data. Descriptive statistics are presented as mean  $\pm$  standard deviation. Continuous variables were analyzed using Mann-Whitney *U* tests. A 2-tailed *P* < .05 was considered statistically significant.

## Results

### Study Population

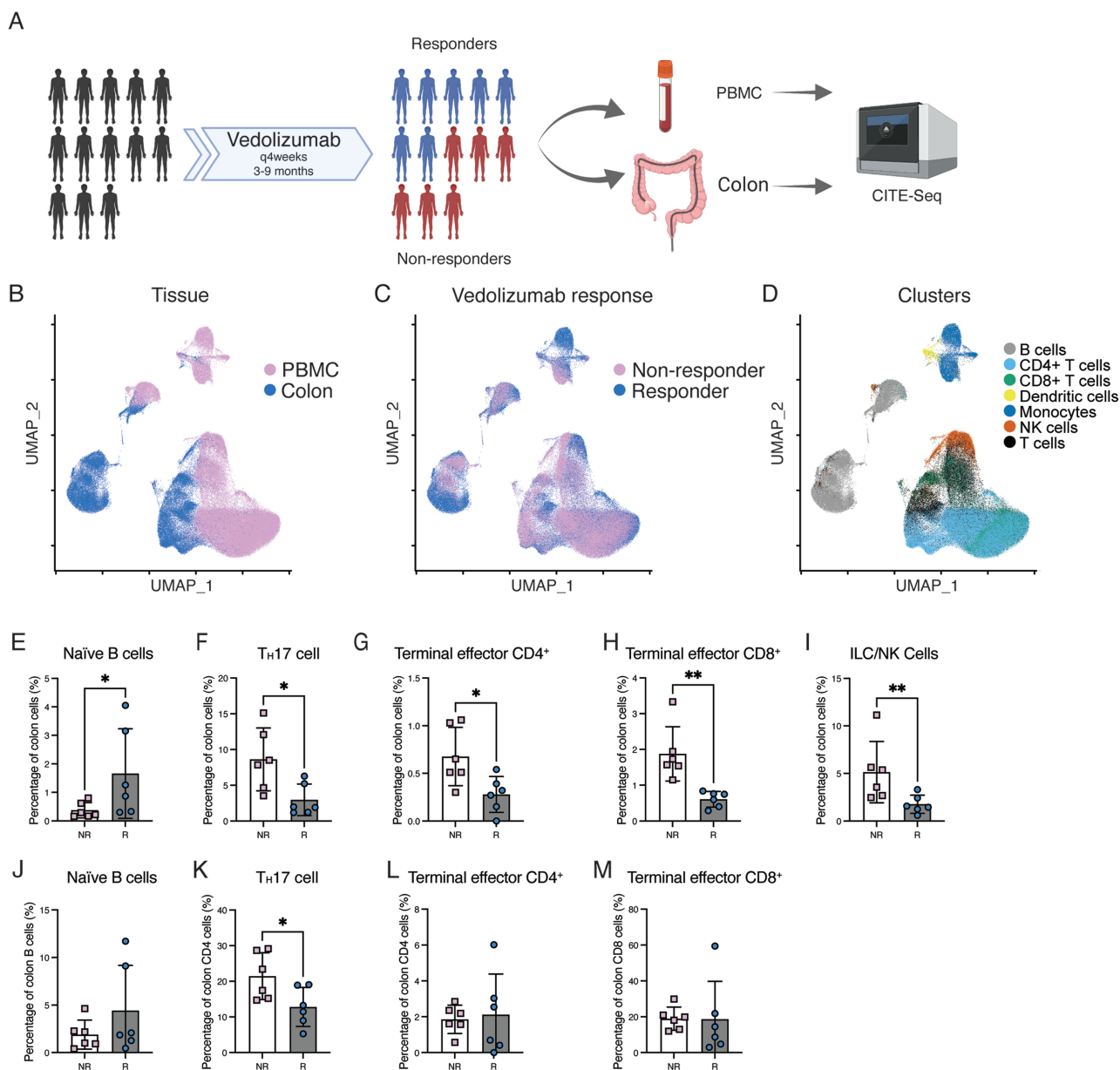
A cohort of 13 patients with ulcerative colitis treated with the anti- $\alpha 4\beta 7$  integrin antibody vedolizumab and evaluated by colonoscopy 5 to 9 months following initiation of drug therapy ([Figure 1A](#), [Supplementary Table 1](#), [Supplementary Table 2](#)) was recruited for the study. Drug response was determined by subsequent endoscopic Mayo score following vedolizumab: 6 patients were categorized as nonresponders with moderate or severe disease, and 7 patients were categorized as responders with no to mild disease. There were 7 females and 6 males, with an approximately equal sex distribution in the nonresponders and responders (57% vs 50%). The mean age was 41.4 years for all patients, 41.5 years for nonresponders, and 41.3 for responders. The mean age at diagnosis was 33.8 years for all patients, 34.5 years for nonresponders, and 33.3 years for responders. The average body mass index was 28.15 for all patients, 25.75 for nonresponders, and 30.21 for responders. With regards to disease extent, 8 of 13 patients (62%) had pancolitis seen on prior endoscopy, 3 of 13 (23%) had left-sided colitis, and 2 of 13 (15%) had proctitis. One of 6 nonresponders was taking concomitant systemic corticosteroids; another nonresponder was taking concomitant methotrexate; and a third nonresponder was taking concomitant azathioprine ([Supplementary Table 2](#)). Two of 7 responders in this cohort were biologic-naïve, but all 6 nonresponders had prior anti-tumor necrosis factor (TNF) therapy with infliximab or adalimumab. The average duration of vedolizumab exposure

before endoscopic evaluation for nonresponders was 228.5 days compared with 153.4 days for responders.

### CITE-seq Analysis of Immune Cells from Vedolizumab Responders and Nonresponders

Cells from mucosal biopsies and peripheral blood were processed into single-cell suspensions, FACS-purified on the basis of CD45, a pan-immune cell marker, and processed for CITE-seq using the 10x Genomics Chromium platform ([Figure 1A](#)). Antibodies targeting 58 proteins were selected for inclusion in the antibody panel ([Supplementary Table 3](#)). Uniform manifold approximation and projection (UMAP) analysis showed that cells from peripheral blood and colon clustered separately ([Figure 1B](#)), whereas cells from nonresponders and responders generally clustered together ([Figure 1C](#)). We broadly annotated the immune cells into 7 major immune cell types and observed that B cells and myeloid cells clustered distinctly from the other immune cell types, including CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells, unconventional T cells, and natural killer (NK) cells ([Figure 1D](#)). To further refine our annotations, we used a previously published set of peripheral blood immune cell transcriptional signatures derived from bulk RNA-seq of FACS-purified cells.<sup>16</sup> Because these immune cell subsets were derived from the peripheral blood rather than tissue, we revised the annotations of these subsets to account for cell populations found in the tissues exhibiting transcriptional signatures similar to those of cells circulating in the peripheral blood ([Supplementary Table 4](#)). For example, the original annotation “NK cells” was revised to “ILC/NK cells,” as NK cells are considered a subset of the innate lymphoid cell (ILC) family and exhibit similar transcriptional signatures.<sup>21</sup>

Immune profiling demonstrated changes in cell abundances in colon tissue but not in the peripheral blood. We observed that T cells were the most abundant immune cell type in the peripheral blood, though there were no differences in the proportions of T cells between responders and nonresponders ([Supplementary Figure 1A](#)). Within colon tissue, responders exhibited an increase in the proportions of cells annotated as naïve B cells compared with that in nonresponders ([Figure 1E](#)), whereas there were no significant changes observed in the proportions of plasmablasts ([Supplementary Figure 1B](#)). Moreover, vedolizumab responders exhibited decreased proportions in T<sub>H</sub>17, terminal effector CD4<sup>+</sup>, terminal effector CD8<sup>+</sup>, and innate lymphoid cells/natural killer cells (ILC/NK cells; [Figures 1F–I](#)), all of which have been previously implicated in the pathogenesis of UC.<sup>22–24</sup> The T<sub>H</sub>1 and T<sub>H</sub>1/17 subsets were not significantly different between responders and nonresponders ([Supplemental Figures 2A and B](#)). There were no significant differences in the proportions of innate immune cells such as myeloid dendritic cells or monocyte subsets within the peripheral blood or colon tissue ([Supplemental Figures 2C–F](#)). However, there was a trend towards increased nonclassical monocytes in responders, corresponding to a decrease in the proportion of classical monocytes as a subset of all monocytes within peripheral blood ([Supplemental Figure 2G, H](#)). Vedolizumab has been previously reported to alter lymphocyte populations within the lamina propria, though no significant alterations in lymphocyte populations in peripheral blood were observed in the current study.<sup>14</sup> The absence of significant alterations to most lymphocyte populations suggested that an alternative mechanism, such as differentiation or cell signaling, may play a role in clinical responders.



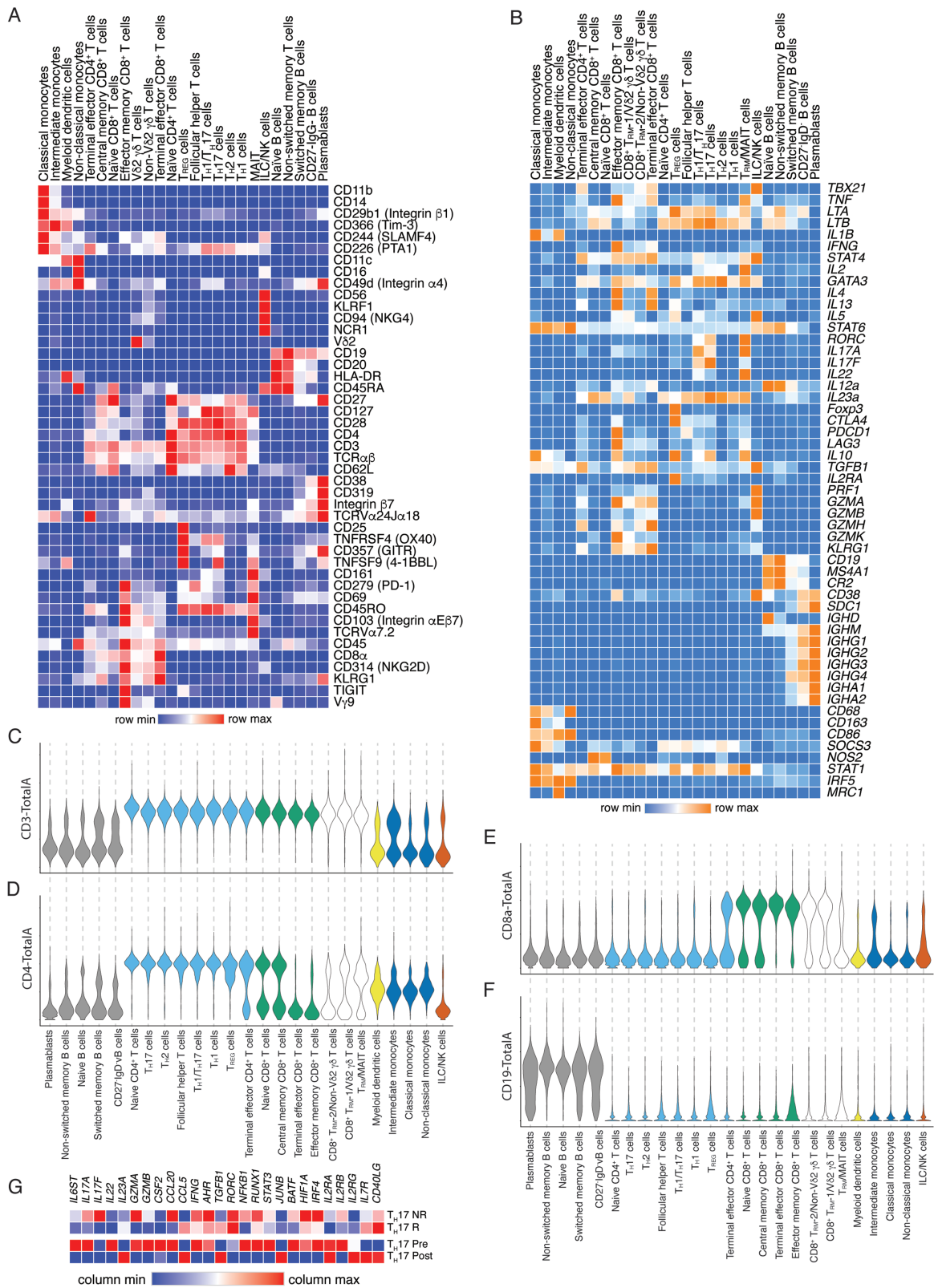
**Figure 1.** CITE-seq analyses of immune cells of vedolizumab nonresponders and responders. A, Experimental design. Immune cells from blood and colon derived from subjects with ulcerative colitis (UC) treated with vedolizumab (VDZ) for 5 to 9 months were stained with the TotalSeq antibody cocktail and fluorescently labeled antibodies, isolated by FACS, and prepared for CITE-seq using the 10x Genomics Chromium platform. Image created with BioRender.com. B, UMAP plots colored by tissue compartment identity, (C) by nonresponder (NR) vs responder (R), and (D) major immune cell type. E-I, Proportions of naïve B cells, T<sub>H</sub>17 cells, terminal effector CD4<sup>+</sup> T cells, terminal effector CD8<sup>+</sup> T cells, and innate lymphoid cell/natural killer cell (ILC/NK) in colon tissue. J, Naïve B cells as a proportion of total colon B cells. K, T<sub>H</sub>17 cells as a proportion of total colon CD4<sup>+</sup> T cells. L, Terminal effector CD4<sup>+</sup> T cells as a proportion of total colon CD4<sup>+</sup> T cells. M, Terminal effector CD8<sup>+</sup> T cells as a proportion of total colon CD8<sup>+</sup> T cells.

We confirmed the accuracy of the modified annotations using the protein and gene expression data (Figure 2A and B). For example, the “tissue-resident memory T cells/MAIT (T<sub>RM</sub>/MAIT)” cluster expressed high levels of CD69 and CD103, which are markers associated with tissue-resident memory T (T<sub>RM</sub>) cells. Cells expressing CD3 were annotated as CD4<sup>+</sup> and CD8<sup>+</sup> T cells and included T<sub>RM</sub> cells (Figure 2C). The 8 clusters annotated as CD4<sup>+</sup> T cells generally had higher expression of CD4 protein (Figure 2D), whereas the 4 clusters annotated as CD8<sup>+</sup> T cells had higher expression of CD8α protein (Figure 2E). The CD19 protein was most highly expressed in

cells annotated as B cells, confirming the accuracy of the annotations (Figure 2F).

### Vedolizumab Responders Exhibit a Decrease in the Inflammatory T<sub>H</sub>17 Cell Effector Program

Interleukin (IL)-17 signaling and T helper 17 (T<sub>H</sub>17) cells have been associated with the pathogenesis and disease activity of UC.<sup>3,22</sup> As mentioned above, vedolizumab responders exhibited a decrease in the proportions of T<sub>H</sub>17 cells compared with nonresponders (Figure 1F). When we compared selected genes expressed by colon T<sub>H</sub>17 cells in the responder, nonresponder, pre-treatment, and post-treatment



**Figure 2.** Expression of canonical genes and proteins in annotated immune cell subsets. **A**, Relative expression of selected proteins included in the CITE-seq antibody panel, represented as a hierarchically clustered summary heatmap; columns represent each of the 25 annotated immune cell subsets, and rows represent individual proteins. **B**, Relative expression of selected canonical genes, represented as a hierarchically clustered summary heatmap; columns represent each of the 25 annotated immune cell subsets and rows represent selected genes. **C-F**, Expression of selected proteins in each of the 25 annotated immune cell subsets, represented as violin plots, ordered, and colored by major immune cell type (gray, B cells; light blue, CD4<sup>+</sup> T cells; green, CD8<sup>+</sup> T cells; black outline, tissue-resident/unconventional T cells; yellow, myeloid cells; blue, monocytes; brown, ILC/NK cells). **G**, Relative expression of selected genes related to T<sub>H</sub>17 function and regulation comparing colon T<sub>H</sub>17 from vedolizumab nonresponders, vedolizumab responders, single subject pretreatment, and single subject post-treatment, represented as a summary heat map; columns represent selected genes and rows represent the samples.

samples (Figure 2G), we observed decreased expression of inflammatory cytokines and granzymes such as *IL6ST*, *IL17A*, *IL17F*, *GZMA*, *GZMB*, *CCL20*, and *IFNG*, consistent with quiescent disease, along with increased expression of *IL23A*, *CCL5*, and *TGFB1*. Similarly, comparison of colon  $T_H17$  cells from the pre- vs post-treatment conditions revealed increased expression of *IL6ST*, *IL17A*, *IL22*, *GZMA*, *GZMB*, *CCL20*, and *IFNG*, along with decreased *IL23A*, *CCL5*, and *TGFB1* expression in pretreatment colon  $T_H17$  cells. With respect to transcription factors involved in  $T_H17$  effector function in nonresponders vs responders, we observed decreased expression of *AHR*, *NFKB1*, *RUNX1*, *STAT3*, *BATF*, *HIF1A*, and *IRF4*. Comparison of cytokine receptor expression revealed increased expression of *IL2RB* (CD122), which controls responsiveness to IL-15, in colon  $T_H17$  cells from vedolizumab responders and the pretreatment sample. Conversely, increased expression of *IL7R* (CD127), which controls responsiveness to IL-7, was observed in colon  $T_H17$  cells from vedolizumab responders and the pretreatment sample. These findings suggest that the clinical response to vedolizumab may be associated with changes in the gene expression and cellular interactions of  $T_H17$  cells.

### $T_H17$ From Vedolizumab Nonresponders Have Increased Signaling From Pro-inflammatory Innate Immune Cells

To explore CCCs that might regulate changes in  $T_H17$  cells in vedolizumab nonresponders vs responders, we applied NicheNet,<sup>17</sup> a previously published computational algorithm that infers ligand-receptor and ligand-target relationships from single-cell gene expression data. The approach involves the following steps: (1) defining the “sender” cell types, “receiver” cell types, and gene set that will guide the selection of prioritized ligands; (2) defining a set of potential ligands expressed by the defined sender cell types known to bind potential receptors expressed by defined receiver cell types; (3) ranking ligands based on transcriptional signatures downstream of the putative ligand; and (4) inferring target genes in receiver cells based on specific ligand-receptor pairs. Thus, putative ligand-receptor pairs can be identified between sender-receiver cell types, and gene expression changes downstream of engagement of ligand-receptor interactions can be assessed.

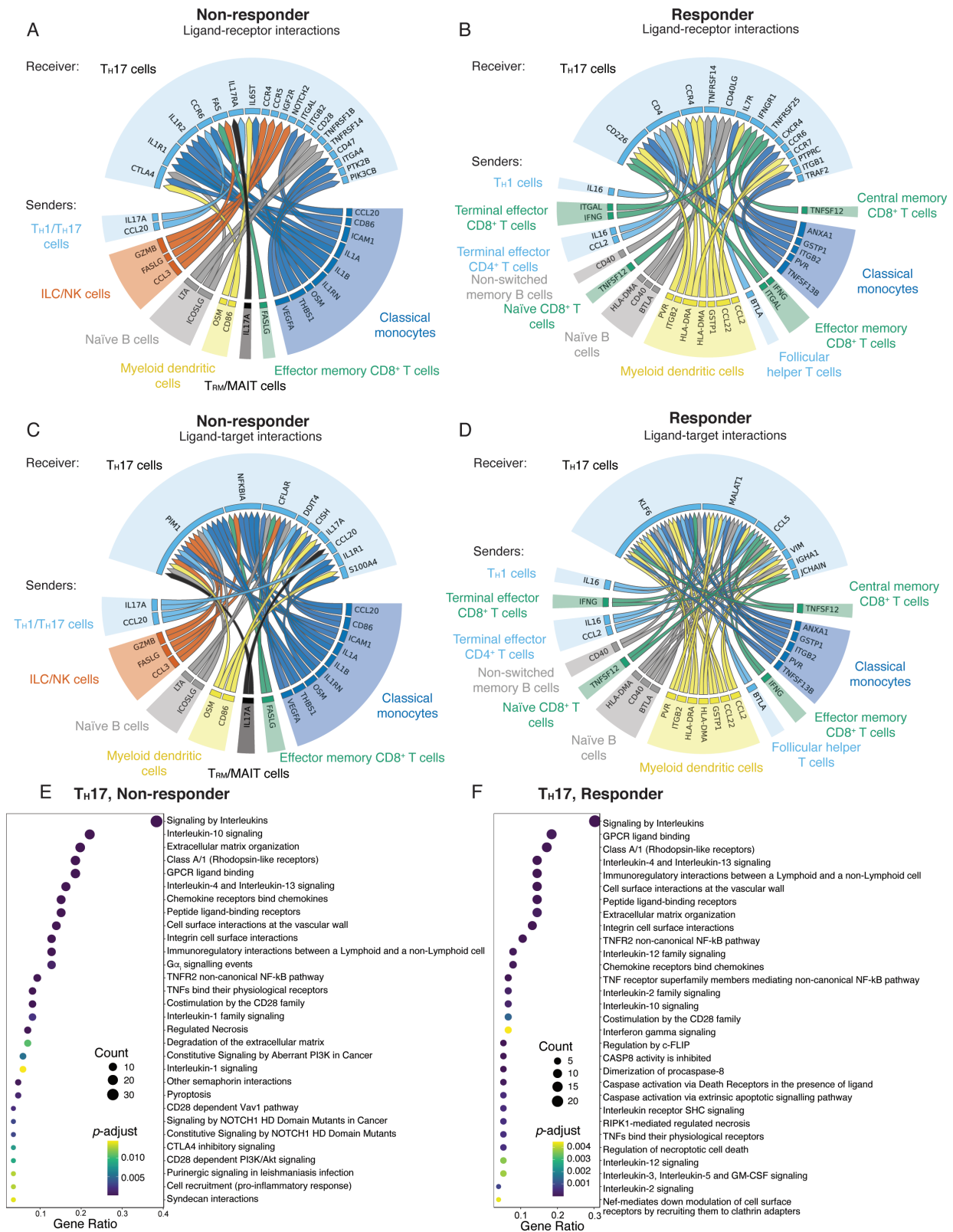
We designated  $T_H17$  cells as receivers and compared ligand-receptor and ligand-target interactions inferred in vedolizumab responders vs nonresponders (Figure 3). We observed that colon  $T_H17$  cells from vedolizumab nonresponders had a greater degree of interactions with classical monocytes compared with responders (Figures 3A, B). Conversely, colon  $T_H17$  cells from vedolizumab responders exhibited more interactions with myeloid dendritic cells compared to nonresponders. Moreover, colon  $T_H17$  cells from vedolizumab nonresponders exhibited interactions with  $T_H1/T_H17$  cells and ILC/NK cells that were strikingly absent in responders, whereas colon  $T_H17$  cells from vedolizumab responders exhibited a number of interactions that were absent in nonresponders, including interactions with follicular T helper cells, naïve B cells, naïve CD8<sup>+</sup> T cells, and central memory CD8<sup>+</sup> T cells (Figures 3A, B).

Focusing next on specific ligand-receptor interactions in vedolizumab nonresponders, we observed that *IL1A* and *IL1B* produced by classical monocytes (Supplemental Figure 3A) were predicted to bind to *IL1RA* and *IL1RB* on colonic

$T_H17$  cells (Figure 3A, Supplemental Figure 3A), suggesting a possible increase in inflammatory interleukin (IL)-1 signaling, which has been associated with active inflammation and  $T_H17$  cell differentiation in the colons of UC patients.<sup>25,26</sup> Expression of oncostatin M (OSM), an IL-6 family cytokine associated with treatment responsiveness, was elevated in both classical monocytes and myeloid dendritic cells.<sup>27</sup> Moreover, *IL17A* was predicted to be produced by  $T_H1/T_H17$  and  $T_{RM}/MAIT$  cells and received by  $T_H17$  cells in nonresponders (Figure 3A, Supplemental Figure 3B). This observation may represent a compensatory response to limit inflammation by  $T_H17$  cells since IL-17A signaling in  $T_H17$  cells has been reported to function in a cell-intrinsic negative feedback loop that limits production of other pathogenic  $T_H17$ -lineage cytokines<sup>28</sup> like IL-17F, IL-22, and GM-CSF (Figure 2G). Lastly, *FASLG* expressed by ILC/NK and effector memory CD8<sup>+</sup> T cells was predicted to bind to *FAS* on  $T_H17$  cells, whereas *GZMB* produced by ILC/NK cells was predicted to bind to its receptor *IGF2R* on  $T_H17$  cells (Figure 3A). These observations raised the possibility of killing of  $T_H17$  cells by ILC/NK cells or effector memory CD8<sup>+</sup> T cells as a compensatory mechanism to dampen inflammation.

We next examined inferred ligand-receptor interactions with colon  $T_H17$  cells in vedolizumab responders. The pleiotropic cytokine interleukin 16, encoded by *IL16*, which has been shown to act as an inhibitor of antigen-specific CD4<sup>+</sup> T cell activation,<sup>29</sup> was expressed by  $T_H1$  and terminal effector CD4<sup>+</sup> T cells and predicted to act on  $T_H17$  cells (Figure 3B, Supplemental Figure 3B), which may result in a dampening of intestinal inflammation. Glutathione S-transferase P, encoded by *GSTP1*, which plays an important role in detoxification by catalyzing the conjugation of many hydrophobic and electrophilic compounds with reduced glutathione, was predicted to be expressed by myeloid dendritic cells; notably, *GSTP1* polymorphisms have been associated with an increased risk of ulcerative colitis.<sup>30</sup> Naïve B cells and  $T_{FH}$  cells exhibited increased expression of B and T lymphocyte attenuator, encoded by *BTLA*, which was predicted to bind to *TNFRSF14*, also known as the herpesvirus entry mediator (HVEM) on  $T_H17$  cells. The complex costimulatory receptor HVEM transmits an activating signal when bound to its ligand LIGHT but an inhibitory signal when bound by *BTLA*; intriguingly, *TNFRSF14* has been identified as an IBD risk gene.<sup>31</sup> Classical monocytes and myeloid dendritic cells produced *ANXA1* (Supplemental Figure 3A), a potent anti-inflammatory protein that has been shown to reduce  $T_H17$  cytokine production<sup>32</sup> and was predicted to bind to *CXCR4* and *CCR6* on  $T_H17$  cells in vedolizumab responders. Taken together, these findings suggest specific CCC in vedolizumab responders that play a role in mediating its effects.

Analyses of predicted gene expression downstream of inferred ligand-receptor interactions in vedolizumab nonresponders revealed induction of a number of genes in colon  $T_H17$  cells. Induction of *IL17A* may reflect uncontrolled inflammation in vedolizumab nonresponders, whereas production of *CCL20* may recruit additional  $T_H17$  cells into the tissue (Figures 3C and 2G).<sup>33</sup> In addition, induction of *IL1R1*, *S100A4*, *DDIT4*, and *PIM1*, factors that promote  $T_H17$  differentiation, may contribute to increased proportions of colonic Th17 cells in vedolizumab nonresponders. Increased interleukin-1 signaling through *IL1R1* may favor the differentiation of  $T_H17$  over  $T_{REG}$  cells by inhibiting TGF $\beta$ -induced



**Figure 3.** NicheNet analyses of colon TH<sub>17</sub> cell interactions with other immune cells in vedolizumab nonresponders vs responders. A-B, Putative ligand-receptor pairs on “sender” immune cells and “receiver” cell (TH<sub>17</sub>), represented as Circos plots, in nonresponders and responders. Colors indicate major immune cell type. Specific immune cell subsets are labeled around the Circos plot. C-D, Genes predicted to be induced in TH<sub>17</sub> cells by predicted ligand-receptor interactions, represented as Circos plots, in nonresponders and responders. E-F, Pathway analyses of enriched genes downstream of predicted ligand-receptor pairs represented as reactome plots in nonresponders and responders.

FOXP3 expression.<sup>26</sup> The calcium binding protein encoded by *S100A4* has been shown to regulate STAT3 activity and expression of the  $T_H17$  lineage-defining factor ROR $\gamma$ t,<sup>30</sup> DNA-damage-inducible transcript 4, encoded by *DDIT4*, has been shown to promote human  $T_H17$  cell differentiation in autoimmune disease.<sup>34</sup> Induction of *PIM1*, which encodes for a serine/threonine kinase, has been shown to promote  $T_H17$  cell differentiation and pathogenicity.<sup>35</sup> In vedolizumab responders, analyses of predicted gene expression downstream of inferred ligand-receptor interactions revealed induction of a distinct set of genes (Figure 3D). For example, *MALAT1* encodes for a long noncoding RNA that has been shown to repress the  $T_H17$  cell effector program and has been associated with reduced disease severity in murine experimental autoimmune encephalomyelitis.<sup>36</sup> Pathway analyses revealed enrichment of pathways involved in IL-1 signaling and pro-inflammatory cell recruitment responses in vedolizumab nonresponders (Figure 3E), whereas enrichment of IL-3 signaling, which has been shown to inhibit  $T_H17$  differentiation and promote  $T_{REG}$  cell differentiation,<sup>37</sup> was enriched in vedolizumab responders (Figure 3F).

### Increased Pro-inflammatory Chemokines and Cytokines Pre- vs Post-therapy

We next performed analyses of a responder patient, corresponding to patient 1 in Supplemental Table 2, for whom we had collected peripheral blood and colon samples prior to and following vedolizumab therapy. We observed decreased proportions of classical and intermediate monocytes along with increased proportions of naïve T cells in the blood following therapy (Supplementary Figure 1C). In the colon, we observed decreased proportions of plasmablasts and increased proportions of  $T_{REG}$  cells (Supplementary Figure 1D). We designated  $T_H17$  cells as receivers and compared ligand-receptor and ligand-target interactions inferred in pre- vs post-therapy samples (Figure 4). Compared with cells from the post-therapy sample, we observed that colon  $T_H17$  cells from the pre-therapy sample exhibited a greater degree of interactions with ILC/NK cells (Figure 4A). By contrast, colon  $T_H17$  cells from the post-therapy sample exhibited more interactions with terminal effector CD8<sup>+</sup> T cells, which was strikingly absent in cells derived from the pre-therapy sample.

Focusing on specific ligand-receptor interactions in the pre-therapy sample, we observed that the chemokines *CCL3* and *CCL20* were predicted to bind to colon  $T_H17$  cells (Figure 4A, Supplemental Figure 3C); these chemokines were notably absent in the post-therapy sample (Figure 4B). In the pre-therapy sample, the pro-inflammatory *FASLG* expressed by CD8<sup>+</sup>  $T_{RM}$ -1/V $\delta$ 2  $\gamma\delta$  T cells and effector memory CD8<sup>+</sup> T cells was predicted to bind to *FAS* on  $T_H17$  cells, whereas *GZMB* produced by ILC/NK cells was predicted to bind to its receptor *IGF2R* on  $T_H17$  cells (Figure 4A). Moreover, the TNF superfamily ligand *LTA* produced by CD8<sup>+</sup>  $T_{RM}$ -1/V $\delta$ 2  $\gamma\delta$  T cells and  $T_{REG}$  cells was predicted to bind to its receptor *TNFRSF1B* (TNFR2) in colon  $T_H17$  cells in the pre-therapy sample (Supplemental Figure 3C); signaling through the inflammatory cytokine *LTA* can induce apoptosis and inflammation, much like TNF.<sup>38</sup> Lastly, *IL22* was predicted to be produced by  $T_H1/T_H17$  cells and  $T_{RM}$ /MAIT cells (Figure 4A, Supplemental Figure 3D). Interleukin-22 signaling, which was elevated in inflamed mucosa, has been shown to stimulate epithelial tissue repair,<sup>39</sup> and its induction in the pre-therapy colon  $T_H17$  cells may represent a compensatory effect to dampen inflammation.

In post-treatment colon samples, *LAMB1*, which encodes for the extracellular matrix protein laminin B1, was expressed by  $T_H2$  cells and predicted to bind to colon  $T_H17$  cells (Figure 4B). Polymorphisms in *LAMB1* have been previously linked to UC risk.<sup>40</sup> The immunoregulatory cytokine *TGFB1* was produced by CD8<sup>+</sup>  $T_{RM}$ -1/V $\delta$ 2  $\gamma\delta$  T cells and effector memory CD8<sup>+</sup> T cells (Supplemental Figure 3C). The homeostatic cytokine *IL7*, which promotes the survival of T cells, was also produced by cells annotated as naïve B cells and predicted to bind to its corresponding receptor complex components *IL7R* and *IL2RG* produced by  $T_H17$  cells. Additional ligand-receptor interactions identified in post-treatment colon  $T_H17$  cells, such as *GSTP1-IL7R* and *IL16-CD4*, were also observed in vedolizumab responders, as described above.

Analyses of pathway analyses of gene expression downstream of inferred ligand-receptor interactions in pretreatment  $T_H17$  cells revealed an enrichment of pathways involved in IL-1 signaling and pro-inflammatory cell recruitment responses, along with signaling of several other cytokines, including IL-12, IL-2, and IL-15 (Figure 4C). Analyses of pathway analyses of gene expression in post-treatment  $T_H17$  cells revealed an enrichment of pathways associated with inhibition of CASP8 activity along with IL-7 signaling (Figure 4D). Taken together, these findings suggest that the clinical response to vedolizumab may be associated with changes in the gene expression and cellular interactions of  $T_H17$  cells and provide a resource data set for the field to investigate other immune cells that may influence the mechanism of action of vedolizumab.

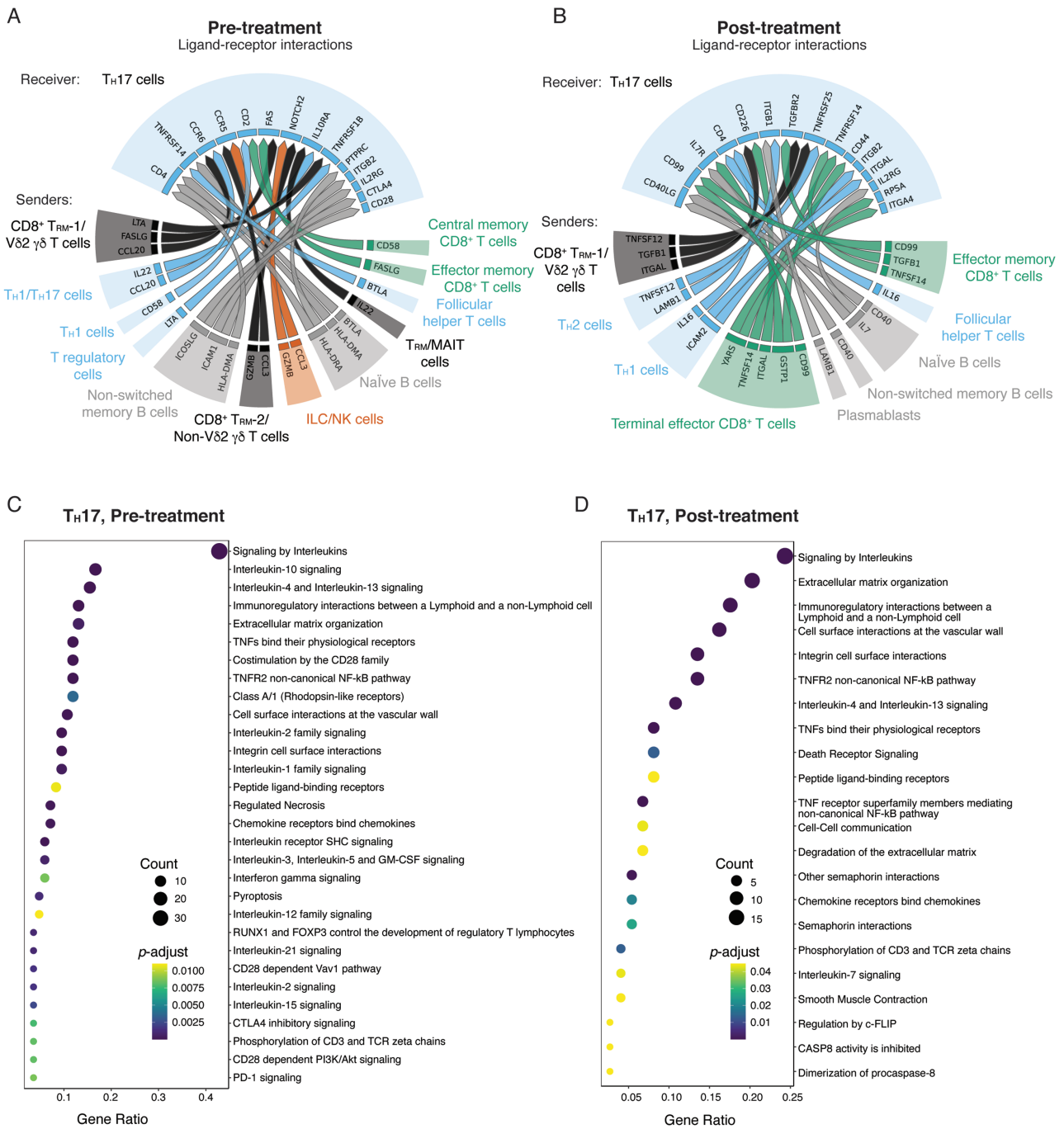
### Discussion

Targeted therapy against  $\alpha$ 4 $\beta$ 7 integrins has been beneficial for IBD patients with a favorable risk-efficacy profile and is mechanistically distinct from TNF and JAK inhibitors and IL-12/23 blockade.<sup>41</sup> The main mechanism of action of vedolizumab is thought to involve blocking  $\alpha$ 4 $\beta$ 7-MAdCAM-1 binding, which limits lymphocyte migration into the gut. However, prior studies have suggested that vedolizumab may also alter gene expression of  $T_H17$  cells and that active UC is associated with increased production of  $T_H17$  cell-associated cytokines.<sup>4,13,14</sup> We hypothesized that  $T_H17$  cells from vedolizumab nonresponders and responders might receive distinct cell signals that could contribute to these previously reported changes. In this study, we bioinformatically inferred CCC using single-cell RNA-sequencing data to identify interactions and signals between  $T_H17$  and other immune cells.

Consistent with previously reported findings, we observed alterations in immune cell populations within colonic tissue in vedolizumab nonresponders vs responders. In particular, we found increased relative proportions of  $T_H17$  cells, terminal effector CD4 and CD8 cells, and ILC/NK cells in nonresponders, along with decreased relative proportions of naïve B cells. This increase in the  $T_H17$  cell compartment in active UC mucosa has been previously reported, though it has not been a consistently identified feature.<sup>3,4,14</sup> We did not observe differences in peripheral immune cell populations between nonresponders and responders.

We found that  $T_H17$  cells in vedolizumab nonresponders exhibited increased expression of  $T_H17$  cell cytokines *IL17A*, *IL17F*, and *IFNG*. This upregulation of an inflammatory IL-17





**Figure 4.** NicheNet analyses of colon T<sub>H</sub>17 cell interactions with other immune cells in a single subject pre- and post-vedolizumab therapy. A-B, Putative ligand-receptor pairs on “sender” immune cells and “receiver” cells (T<sub>H</sub>17), represented as Circos plots, in pre-treatment and post-treatment samples. Colors indicate major immune cell type. Specific immune cell subsets are labeled around the Circos plot. C-D, Pathway analyses of enriched genes downstream of predicted ligand-receptor pairs, represented as reactome plots, in pre-treatment and post-treatment samples.

response has been reported in active UC tissue compared with noninflamed and healthy tissue<sup>3</sup> and is likely a reflection of decreased disease activity.

Although we did not observe differences in the proportions of innate immune cell subsets in gut tissues of vedolizumab nonresponders and responders, we found differences in T<sub>H</sub>17 cell-associated signaling pathways. In vedolizumab nonresponders, we identified inflammatory monocytes/macrophages expressing *IL1A*, *IL1B*, *IL1RN*, *OSM*, and *CCL20*; *OSM*-expressing myeloid dendritic cells; and ILC/

NK cells expressing *GZMB*, *FASLG*, and *CCL3*. Reduction of pretreatment expression levels of *OSM* following TNF inhibition or vedolizumab has been recognized as a marker of treatment responsiveness<sup>27,42</sup>; the elevated *OSM* expression in the nonresponders observed in the current study is likely a reflection of persistent disease activity and a failure to adequately suppress *OSM*. Ligand-target analysis identified *PIM1* and *IL1R1*, factors that promote T<sub>H</sub>17 cell differentiation, as well as *CCL20*, a chemokine that recruits additional T<sub>H</sub>17 cells. Expression of *PIM1* has been reported in pro-inflammatory

$T_H17$  cells and is necessary for  $T_H17$  cell differentiation in experimental models of uveitis.<sup>35</sup> Additionally, IL-1 signaling has been shown to promote  $T_H17$  cell differentiation and expansion,<sup>26</sup> and increased IL-1 signaling from pro-inflammatory macrophages has also been demonstrated in other studies.<sup>3,13</sup> The signaling environment for vedolizumab responders was notably less inflammatory and reflected genes and pathways upregulated during homeostasis. For example, *ANXA1*, which is associated with resolution of inflammation and decreased lymphocyte migration,<sup>32</sup> and *MALAT1*, which has been shown to inhibit inflammatory macrophage polarization and  $T_H17$  cell differentiation,<sup>36</sup> were expressed in more highly expressed in vedolizumab responders. Thus, innate immune cells in vedolizumab nonresponders appear to express molecules that promote  $T_H17$  cell differentiation, expansion, and retention that may contribute to the observed increased  $T_H17$  cell proportions and activity. Taken together, the data raise the possibility that vedolizumab may decrease inflammatory  $T_H17$  cell activity indirectly through effects on innate immune cells.

All of the nonresponders had prior TNF therapy, but only 2 of the responders were biologic-experienced (Supplemental Table 2). Although not statistically significant, this trend is consistent with the clinical observation that previous exposure to biologics is associated with poorer response to subsequent types of therapy and raises the possibility that the observations reported here may have been influenced by prior immunosuppressive therapy. Other groups have observed that responders to anti-TNF therapy exhibited decreased monocyte numbers and activation compared with nonresponders,<sup>43,44</sup> though the long-term durability of changes following discontinuation of anti-TNF therapy on immune profiles has not been established. In our cohort, we observed that within the peripheral monocyte compartment of vedolizumab nonresponders, there was a greater proportion of classical monocytes even after previously having received anti-TNF therapy (Supplemental Figure 2G). This supports the notion that a differential monocyte response is a marker of drug responsiveness for both anti-TNF therapy and vedolizumab.<sup>45</sup> While the conventional synthetic disease modifying anti-rheumatic drugs methotrexate and azathioprine also have broad immunosuppressive effects on monocyte maturation and function, they appeared to be ineffective in the nonresponders who still exhibited evidence of inflammatory macrophage and dendritic cell gene expression profiles (Supplemental Figure 3A). Our study was not designed to detect differences in absolute cell number or other drug-specific effects.

There were several limitations of the study. Our study cohort was small and had limited representation from diverse ethnic backgrounds; as such, we were unable to draw any significant conclusions regarding drug response and immune profile based on ethnic backgrounds. Furthermore, our cohort lacked non-vedolizumab-treated patients in remission, limiting our ability to distinguish whether the changes we observed were a direct consequence of vedolizumab therapy or simply due to reduced UC disease activity. There was only 1 patient with paired pre- and post-treatment samples, precluding us from inferring cause and effect of vedolizumab therapy. Indirect comparison of our findings with a previously published set of differentially expressed genes in colonic tissue prior to vedolizumab treatment<sup>42</sup> showed minimal overlap, with the exception of increased CD40L expression in responder  $T_H17$  cells (Figure 2G); as noted previously, nonresponders in the current study had increased expression

of *OSM* in contrast to the decreased *OSM* expression seen in vedolizumab nonresponders in a previously published data set.<sup>42</sup> As our study also did not analyze nonimmune cells, we were unable to infer contributions from nonimmune cells in the colonic tissue, thereby narrowing the scope of our investigation of CCC, and we were unable to make direct comparisons to prior studies using bulk tissue. Future investigations with mechanistic studies will be needed to further elucidate the interactions between  $T_H17$  and innate immune cells in UC and whether vedolizumab acts through mechanisms that are independent of lymphocyte trafficking.

## Supplementary Data

Supplementary data is available at *Inflammatory Bowel Diseases* online.

## Author Contributions

Conceptualization: B.S.B., J.T.C.; investigation and analysis: P.H., E.Y.C., C.S.I., P.Y., Y.L., S.A.V., W.H.W., J.G.O., B.S.B., M.S.T.; supervision and funding acquisition: W.W., J.T.C.

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## Conflicts of Interest

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## Data Availability

The data sets generated for this study are available at Gene Expression Omnibus under accession number [GSE228165](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE228165).

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