



BASIC SCIENCE ARTICLE

Patterns of mucosal inflammation in pediatric inflammatory bowel disease: striking overexpression of IL-17A in children with ulcerative colitis

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BACKGROUND: Aberrant immune responses play a key role in the pathogenesis of inflammatory bowel disease (IBD). Most studies conducted to delineate the underlying molecular mechanisms focus on adults; an understanding of these mechanisms in children remains to be determined. Here, cytokines and transcription factors produced by immune cells within the intestinal mucosa of pediatric patients stricken with ulcerative colitis (UC) and Crohn's disease (CD) are characterized; potential diagnostic and therapeutic targets are identified.

METHODS: Fifty-two pediatric IBD and non-IBD patients were enrolled in the study. Specimens were taken during ileocolonoscopy. Expression of 16 genes that encode cytokines or transcription molecules was determined by quantitative polymerase chain reaction. Clinical data were collected via retrospective chart review.

RESULTS: Overexpression of interleukin-17A (IL-17A) was evident in children with UC compared to both non-IBD and CD patients. IL-22 was strongly increased in UC patients only. Typical proinflammatory and immunoregulatory cytokines were pronounced in IBD patients, although to a lower extent in the latter case. Clustered gene expression enabled differentiation between UC and non-IBD patients.

CONCLUSION: Our findings highlight the crucial involvement of IL-17A immunity in the early course of IBD, particularly UC, and the potential value of gene panels in diagnosing pediatric IBD.

Pediatric Research (2020) 87:839–846; <https://doi.org/10.1038/s41390-019-0486-5>

INTRODUCTION

Inflammatory bowel disease (IBD) is a relevant cause of morbidity afflicting over 2.5 million people and costing approximately 5 billion euros in direct annual health costs in Europe alone.¹ The two principal forms of IBD: ulcerative colitis (UC) and Crohn's disease (CD), share many disease symptoms, but generally differ in the sites of inflammation along the digestive tract.² Approximately 25% of patients with IBD are first diagnosed during childhood or adolescence.³ At this age, the effects of IBD often extend far beyond its physical manifestations; therapeutic options remain unsatisfactory in many cases. Distinguishing disease entities in pediatric IBD is complicated by a high percentage of overlapping or atypical presentations. This often leads to mislabelling and an initial, unclassified IBD (IBD-U) in 7.7% of patients.^{4,5} Consequently, improved treatments and parameters to distinguish disease entities in children are needed.

A variety of factors (immunological, as well as infectious, genetic, and environmental) contribute to the dysregulation of mucosal immunity in the intestinal tract and the development of IBD.^{6,7} Disruption of both the innate and adaptive immune responses, the altered production of a variety of pro- and anti-inflammatory cytokines, and, consequently, an imbalanced cytokine profile are involved. Intestinal epithelial cells,

macrophages, dendritic cells, natural killer cells, helper T cell subsets (i.e., T-helper type 1 (T_H1), T_H2, and T_H17) and regulatory T (T_{reg}) cells play key roles in cytokine production and the pathogenesis of IBD.^{2,7,8} The balance between different T cell lineages is altered in IBD; cell signaling and function differ in cases of CD and UC.^{2,8} CD is characterized by the predominant production of T_H1-type cytokines, such as tumor necrosis factor- α (TNF- α), interferon- γ (IFN- γ), and interleukin-6 (IL-6), which promote IL-12 and IL-23A release by dendritic cells and macrophages. UC, on the other hand, is distinguished by the production of T_H2-type cytokines and, to a lesser extent, IFN- γ .⁷

In addition to T_H1 and T_H2 cell subsets, the number of T_H17 cells is elevated in the sera and intestinal mucosa of IBD patients.⁹ T_H17 cells produce IL-17A, IL-17F, and IL-22 (among other cytokines), which can contribute to intestinal inflammation.² Active IBD is associated with a decrease of forkhead box P3 (FOXP3)⁺CD4⁺ T_{reg} cells in peripheral blood, but a moderate expansion of the same T cell population in the inflamed intestinal mucosa.¹⁰ Given the immunosuppressive activity generally attributed to T_{reg} cells, it is often assumed that their accumulation in these inflamed tissues serves an anti-inflammatory function. Indeed, the production of IL-10 and transforming growth factor- β 1 (TGF- β 1) by T_{reg} cells in experimental mouse models of colitis exerts a significant protective effect.¹¹ Ironically, T_{reg} cells accumulated in the

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Received: 19 October 2018 Revised: 13 June 2019 Accepted: 18 June 2019

Published online: 1 July 2019

intestinal mucosa of patients with active UC and CD fail to suppress inflammation.^{7,10}

Most studies undertaken to date have focused upon the immunological factors that contribute to IBD in adults. Despite the fact that the immune system is subject to dynamic changes throughout life, comparatively little is known about these same factors in the large and growing population of pediatric patients. The demographic data, clinical features, and the pathogenesis of IBD differ markedly depending upon the age of disease onset.¹² As such, it is questionable to what extent the results of studies that focus on adults can be transferred directly to a pediatric setting.

The aim of the present study is to improve the understanding of early immune responses in cases of UC and CD by characterizing and comparing the immunological patterns expressed in the intestinal mucosa of mostly treatment-naive children and adolescent patients. Delineating potential new diagnostic and therapeutic targets is an immediate goal.

METHODS

Tissue sampling and specimen processing

From 2010 to 2017 intestinal tissue biopsies were collected during ileocolonoscopy from children and adolescents (5–18 years of age) with suspected or confirmed IBD and non-IBD. Up to six samples were taken either from sites of macroscopic inflammation if present (IBD patient) or from areas without signs of inflammation (non-IBD patient). No samples were taken from the healthy tissue of IBD patients with macroscopic inflammation. Diagnosis of UC, CD, or non-IBD was based upon clinical, endoscopic, and pathohistological presentation. Patients with unclassified colitis were excluded from the study. Specimens were fixed separately in Allprotect Tissue Reagent (Qiagen, Hilden, Germany) and stored at -80°C . Clinical data concerning patient demographics, laboratory values, clinical presentation, and treatment were collected via retrospective chart review. The study design was approved by the ethics committee of the State Chamber of Physicians of Rhineland-Palatinate [Ethikkommission der Landesärztekammer Rheinland-Pfalz, reference number 837.513.10 (7522)]. Written informed consent was obtained from each patient of legal age or their legal guardian.

Isolation and measurement of RNA

RNA was isolated using the RNeasy Mini Kit and RNase-free DNase Set (both Qiagen) according to the manufacturer's protocol, which included tissue homogenization with a TissueLyser II (Qiagen). Different samples derived from a single individual were isolated separately. The purity and concentration of isolated RNA were determined with NanoDrop[®] ND-1000 (PEQLAB, Erlangen, Germany).

Reverse transcription and quantitative polymerase chain reaction RNA (0.5 μg) purified from each sample was reverse transcribed into complementary DNA (cDNA) using the QuantiNova Reverse Transcription Kit (Qiagen) according to the manufacturer's protocol. Following reverse transcription (RT), cDNA from multiple specimens derived from an individual were pooled. Quantitative polymerase chain reaction (qPCR) was performed on a LightCycler[®] 2.0 in triplicates using LightCycler[®] FastStart DNA MasterPLUS SYBR Green I (both Roche Diagnostics, Mannheim, Germany) according to established standard protocols (45 cycles of amplification, consisting of denaturation at 95°C for 10 s, annealing at 60°C for 10 s, and elongation at 72°C for 5 s). Gene expression was measured as cycle of quantification (C_q), and differences between expression of each target (mean of triplicate result) and the mean of two reference genes were calculated as ΔC_q values. If gene expression was below the detection level, an artificial value half of the lowest measured value was considered

instead as described by Hölttä et al.¹³ Selection of targeted genes assessed was based upon thorough search of the literature [Supplemental Fig. S1 (online)]. Primers for the following target genes: *TNF- α* , *IFN- γ* , *IL-6*, *IL-10*, *IL-12A*, *IL-12B*, *IL-17A*, *IL-22*, *IL-23A*, *TGF- β 1*, SMAD family member 7 (*SMAD7*), T-box 21 (*TBX21*), GATA binding protein 3 (*GATA3*), Spi-1 proto-oncogene (*SPI1*), RAR-related orphan receptor C (*RORC*), *FOXP3*, and reference genes: glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), ribosomal protein lateral stalk subunit P0 (*RPLP0*) were validated beforehand by qPCR and agarose gel electrophoresis. Efficiency was determined by serial dilution qPCR for primers ordered by sequence. Primer sequences and ordering numbers are provided in Supplemental Table S2 (online).

Patient selection for statistical analysis

mRNA isolated from 62 patients was processed initially. For statistical analysis, ten patients were excluded retrospectively due to insufficient quality of probes (defined as C_q for reference genes above 27), leaving 15 patients with UC, 12 patients with CD, and 25 non-IBD children included in the study [for precise patient selection pathway and exclusion criteria, see Supplemental Fig. S3 (online)].

Statistical analysis

Using the $2^{-\Delta C_q}$ formula,¹⁴ the measured ΔC_q values were brought into a linear form. For each gene, the fold change was calculated as the quotient of the mean (normally distributed data) or median (non-normally distributed data) from the IBD and non-IBD groups, respectively. Statistical analysis was performed with IBM SPSS Statistics 23.0 (IBM, Armonk, NY, USA) using analysis of variance and Scheffé test for normally distributed data and Kruskal–Wallis and Mann–Whitney *U* test for non-normally distributed data. Since there was no formal adjustment for the number of performed tests, *p* values should be considered to be exploratory means. Unsupervised hierarchical clustering based upon measured gene expression levels was conducted with R 3.4.1 Pheatmap Package.¹⁵ Graphs were created with GraphPad Prism 7 (GraphPad Software, La Jolla, CA, USA) and R 3.4.1 Pheatmap Package.¹⁵

RESULTS

Study cohort

Demographics, laboratory results, and clinical presentation of the study cohort are detailed in Table 1. Nineteen out of 27 IBD patients were newly diagnosed at the time of sample collection; 16 of these patients were naive regarding treatment with systemic anti-inflammatory or immunosuppressive drugs (in the pre-treated group, prescribed agents included corticosteroid, azathioprine, mesalazine, and ciclosporin). Gender ratio and mean of age were similar between the groups. CD patients showed elevated values for C-reactive protein and anti-*Saccharomyces cerevisiae* antibodies. A higher percentage of perinuclear anti-neutrophil cytoplasmic antibodies was observed in UC patients. Hemoglobin levels were decreased in both IBD groups compared to negative controls. None of the pre-treated patients had received anti-TNF- α or anti-p40-subunit antibody.

Immunological findings

Eleven of the 16 targeted genes assessed showed a statistical difference ($p < 0.05$) in the mRNA expressed in tissues derived from IBD patients compared to the negative controls (Fig. 1). IL-17A mRNA was sharply and consistently elevated in UC patients relative to both non-IBD (71-fold) and CD patients. IL-22 mRNA was strongly increased (17-fold) in UC patients only. Among the inflammatory cytokines that typify T_H1 -type immune responses, the expression of both IL-6 and IFN- γ was pronounced in UC and CD patients, TNF- α mRNA expression, however, was elevated only

Table 1. Study cohort

	Non-IBD	UC	CD
Sample size	25	15	12
Patient demographics			
Age at biopsy (years)	12.1 (± 4.1)	13.7 (± 2.6)	13.4 (± 2.6)
Age at first diagnose (years)		12 (10, 15)	11.5 (11, 13)
Sex, f:m	14:11	8:7	6:6
Laboratory values ^a			
CRP (mg/l)	0.5 (0.1, 1.3)	4.9 (1.1, 12)	25 (4.2, 48.3)
Hemoglobin (g/dl)			
f	13.4 (12.3, 14.3)	11.7 (9.6, 12.3)	11.3 (9.5, 12.5)
m	14.2 (13.4, 15.8)	12.9 (9.3, 13.9)	11.3 (10.6, 13.2)
pANCA positive	0 (0)	6 (42.9)	2 (18.2)
IgG ASCA (U/ml)	7.1 (5.4, 21.9)	7.4 (4.4, 11.6)	71.8 (15.2, 101.1)
IgA ASCA (U/ml)	6.2 (3.5, 11.9)	9.1 (4.9, 13.5)	49.8 (12.8, 210)
Clinical presentation ^a			
Abdominal pain	16 (69.6)	6 (40)	9 (75)
Diarrhea	6 (26.1)	13 (86.7)	8 (66.7)
Blood-positive stool	17 (73.9)	13 (86.7)	3 (25)
Weight loss	2 (8.7)	7 (46.7)	3 (25)
Systemic medication ^b			
At the time of biopsy	0 (0)	4 (26.7)	5 (41.7)
Previously	0 (0)	5 (33.3)	5 (41.7)

IBD inflammatory bowel disease, CRP C-reactive protein, Ig immunoglobulin, ASCA anti-*Saccharomyces cerevisiae* antibodies, pANCA perinuclear anti-neutrophil cytoplasmic antibody, UC ulcerative colitis, CD Crohn's disease, TNF- α tumor necrosis factor- α , IL interleukin

^aData for laboratory values and clinical presentation were not available from all patients; a minimum of 43 patients (82.7% of the cohort) is included for each parameter shown. Means (\pm standard deviation) are shown for normally distributed data; medians (1. quartile, 3. quartile) are shown for non-normally distributed data. Dichotomous variables are displayed as n (%)

^bTreatment status was evaluated depending on the administration of systemic immunosuppressive or anti-inflammatory medication previously or at the time of biopsy. Agents used to treat the pre-treated group included corticosteroids, azathioprine, mesalazine and ciclosporin. None of the patients received immunotherapy (e.g., anti-TNF- α or anti-IL-12/23 antibody)

in mucosal tissues derived from CD patients. A slight difference in IL-12A mRNA expression was observed in tissues derived from control and IBD patients, while IL-12B mRNA was below the levels of detection in most samples. On the other hand, IL-23A (a member of the IL-12/IL-23 axis) was overexpressed in tissues derived from both patient groups. The level of mRNA encoding TGF- β 1, a T_{reg} cell-associated cytokine found often to exert a protective function in experimental animal models, was increased slightly in tissues derived from CD patients. The levels of IL-10 mRNA, a second cytokine with anti-inflammatory activity, were equivalent in control, UC, and CD tissues. Messages encoding transcription factors TBX21 (promotes T_H1 cell function), SPI1 (induces macrophage activation/differentiation), and FOXP3 (stimulates T_{reg} cell development and function) were elevated in the tissues of pediatric UC and CD. While elevated, FOXP3 mRNA

levels in the CD group were extremely variable and not substantially different from the other two groups. No statistical difference between all three groups could be demonstrated in the expression of GATA3 (regulates T_H1 and T_H2 cell differentiation) and RORC (promotes T_H17 cell differentiation) mRNAs. Comparing gene expression in the inflamed mucosa of UC vs. CD patients directly, only TNF- α and IL-17A mRNA levels differed markedly. Higher levels of TNF- α and IL-17A messages were found in the tissues of CD and UC patients, respectively. Fold changes in the expression of all the targeted genes measured are illustrated in Fig. 2.

Since not all patients enrolled in this study were treatment-naive at the time of biopsy, further analysis was performed comparing non-IBD and untreated IBD children only. Exclusion of pre-treated children did not change statistical differences in the expression of the vast majority of targeted genes (representative data shown in Fig. 3a). In contrast to these genes and the representative data shown, TNF- α message expression was elevated only in tissues derived from the CD-treated, not untreated, children relative to the negative control (Fig. 3b).

Heatmap and gene expression patient clusters

Unsupervised hierarchical clustering revealed the segregation of patients into four groups (Fig. 4). An aggregation of diagnoses was clearly visible for UC and non-IBD patients; cluster 1a comprised 80% of non-IBD patients and cluster 2b 80% of UC patients. The results were even more pronounced when the patients were grouped into two main clusters: 100% of non-IBD patients in the first cluster and 87% of UC patients in the second. In contrast, CD patients displayed a random distribution.

DISCUSSION

The pediatric IBD cohort described herein displayed the consistent overexpression of IL-17A cytokine mRNA in tissues derived from UC patients and, to a lesser extent, patients with CD. The expression of IL-22 message, which similarly derives from T_H17 cells, was also elevated markedly in UC, relative to CD, patient tissues. These findings support the potential contribution of T_H17 cells to the pathogenesis of pediatric UC. Rosen et al.¹⁶ also reported increased levels of IL-17A and IL-22 messages expressed in mucosal tissues obtained from pediatric IBD patients; message levels tended to be higher in UC, compared to, CD patients. Hölttä et al.¹³ also found increased concentrations of both IL-17A and IL-22 mRNAs in the tissues of a small cohort of pediatric UC and CD patients; these concentrations differed significantly from the controls, but not from each other. Both Zhu et al.¹⁷ and Elshal et al.¹⁸ also described significantly higher IL-17A mRNA levels in pediatric IBD patients, and elevated percentages and absolute numbers of CD4⁺IL17A⁺ cells in peripheral blood, which occurred most prominently in cases of UC. Taken together, these findings support the potential contribution of T_H17 cells to pediatric IBD, and to UC in particular. Moreover, they concur with the results of studies conducted with adults in which the number of T_H17 cells and the expression of IL-17A and IL-22 mRNAs were increased in IBD patients compared to control individuals.^{19–22} These increases in adults correlated with disease activity, albeit only inconsistent differences between UC and CD patients were found.

The roles of T_H17 cells and IL-17A in immunity are often viewed as a double-edged sword exhibiting both positive and negative activities. In contrast to its purported role in the pathogenesis of IBD, for example, IL-17A can exert tissue-protective effects on the epithelial barrier dependent mostly upon immune cell recruitment (i.e., neutrophil).²³ This duality in function is clearly demonstrated by the positive effects of anti-IL-17A antibody (secukinumab) treatment on plaque psoriasis, which is characterized by elevated IL-17A message in the lesions.²⁴ CD patients treated with anti-IL-17A or anti-IL-17A receptor antibody (brodalumab), on the other

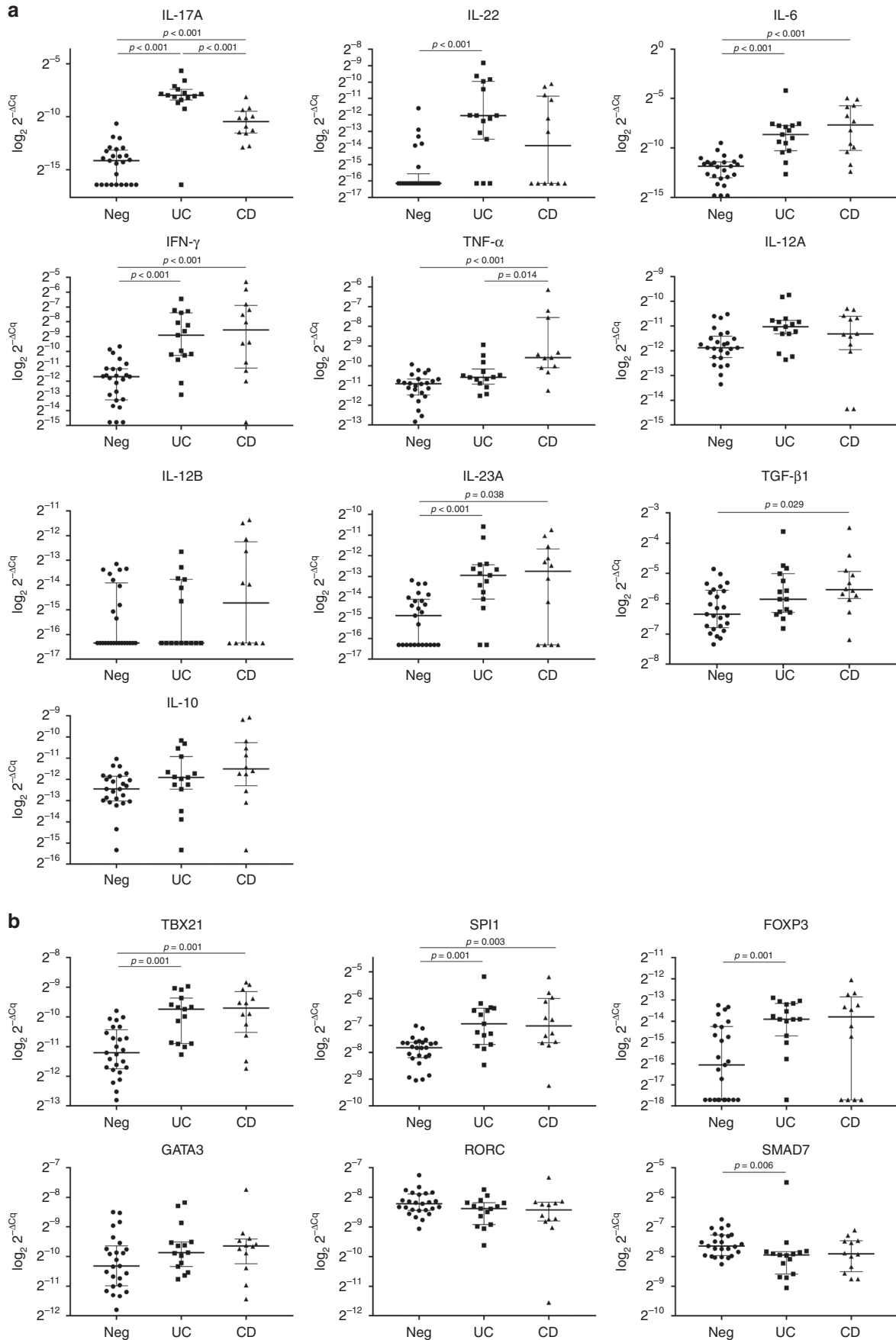


Fig. 1 Gene expression in mucosal specimens measured by quantitative polymerase chain reaction (qPCR). **a** Cytokines. **b** Transcription factors. For each patient and gene, qPCR was performed in triplicates and the mean C_q result was used for further analysis. Data are the logarithmized $2^{-\Delta C_q}$ values displayed as dots. Median and interquartile range are shown within the groups

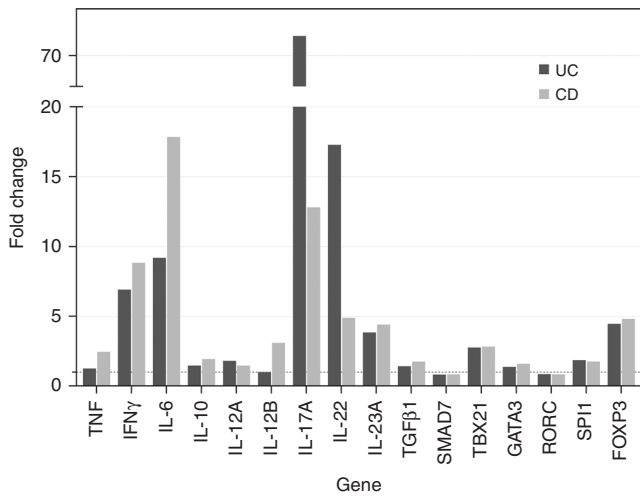


Fig. 2 Fold change in mucosal gene expression. Fold change was calculated from quantitative polymerase chain reaction (qPCR) gene expression data as the quotient of the mean (normally distributed data) or median (non-normally distributed data) measured in the inflammatory bowel disease (IBD) and non-IBD group, respectively. Data obtained for the entire study cohort (52 patients) were used for analysis

hand, had much poorer outcomes in clinical trials than did patients treated with a placebo.^{25,26} These results suggest that IL-17A may actually exert a protective effect in cases of IBD, albeit its precise role remains to be determined. The increased IL-17A gene expression found in pediatric IBD patients studied here could represent a response to, rather than the cause of, bowel inflammation. As such, T_H17 cell-mediated protection rather than blocking IL-17A offers an alternate line of investigation and a potential avenue of treatment.

RORC encodes a major transcription factor (ROR γ t) essential for T_H17 cell differentiation and function.²³ The SMAD7 protein product suppresses the negative response of T_H17 cells to TGF- β 1 and, consequently, the immunosuppressive effect of T_{reg} cells.²⁷ Ironically, both RORC and SMAD7 message levels were equivalent in mucosal tissues derived from groups of control, UC, and CD patients, despite marked differences in IL-17A and IL-22 mRNA expression found in these same tissues. Conceivably, post-translational modifications of the protein products (i.e., ROR γ t and Smad7) accounts for this discrepancy and enables the elevated production of cytokine messages by T_H17 cells in the absence of increased RORC and SMAD7 expression.²⁸ This speculation is supported by results demonstrating increased concentrations of Smad7 protein, but equivalent levels of SMAD7 mRNA, in the tissues obtained from UC and CD patients relative to the normal control group.²⁹

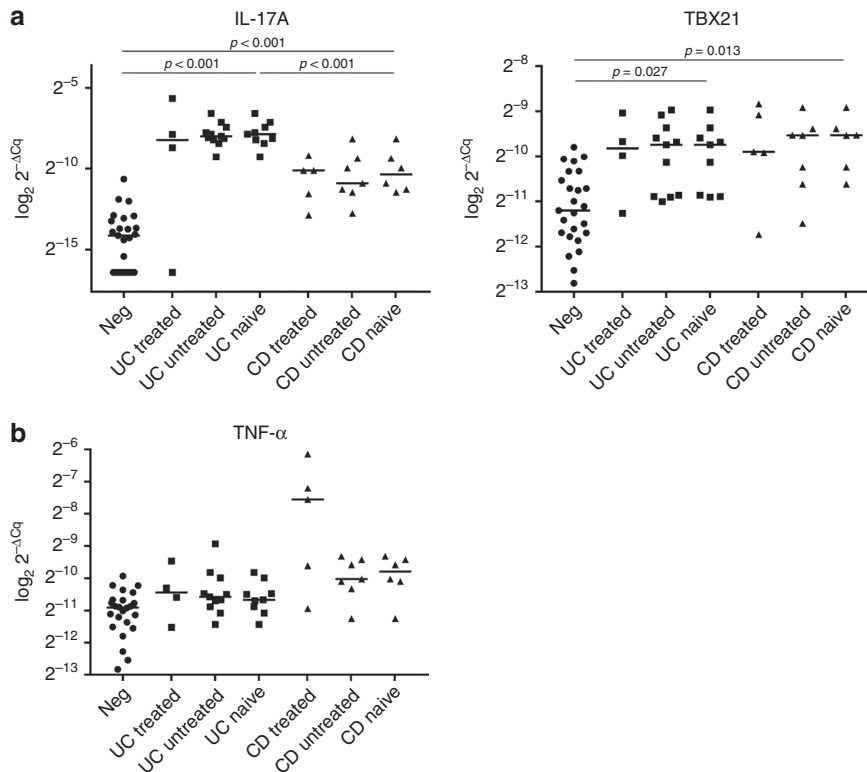


Fig. 3 Influence of pre-treatment on gene expression in mucosal specimen obtained from inflammatory bowel disease (IBD) patients. The ulcerative colitis (UC) and Crohn's disease (CD) groups of patients were divided into those individuals who received systemic IBD treatment ("treated") and those who did not ("untreated") at the time of sample biopsy. "Naive" individuals were patients in the untreated groups who never received systemic IBD treatment. Data analysis and presentation were performed in accordance with Fig. 1, omitting interquartile ranges due to small sample sizes. *P* values <0.05 confirming statistical differences between non-IBD (negative) individuals and treatment-naive patients are shown. **a** The results shown for interleukin-17A (IL-17A) and T-box 21 (TBX21) are representative. **b** Exclusion of pre-treated patients from the analysis of tumor necrosis factor- α (TNF- α) gene expression resulted in *P* values >0.05 comparing non-IBD and CD patients. None of these pre-treated patients received anti-TNF- α antibody

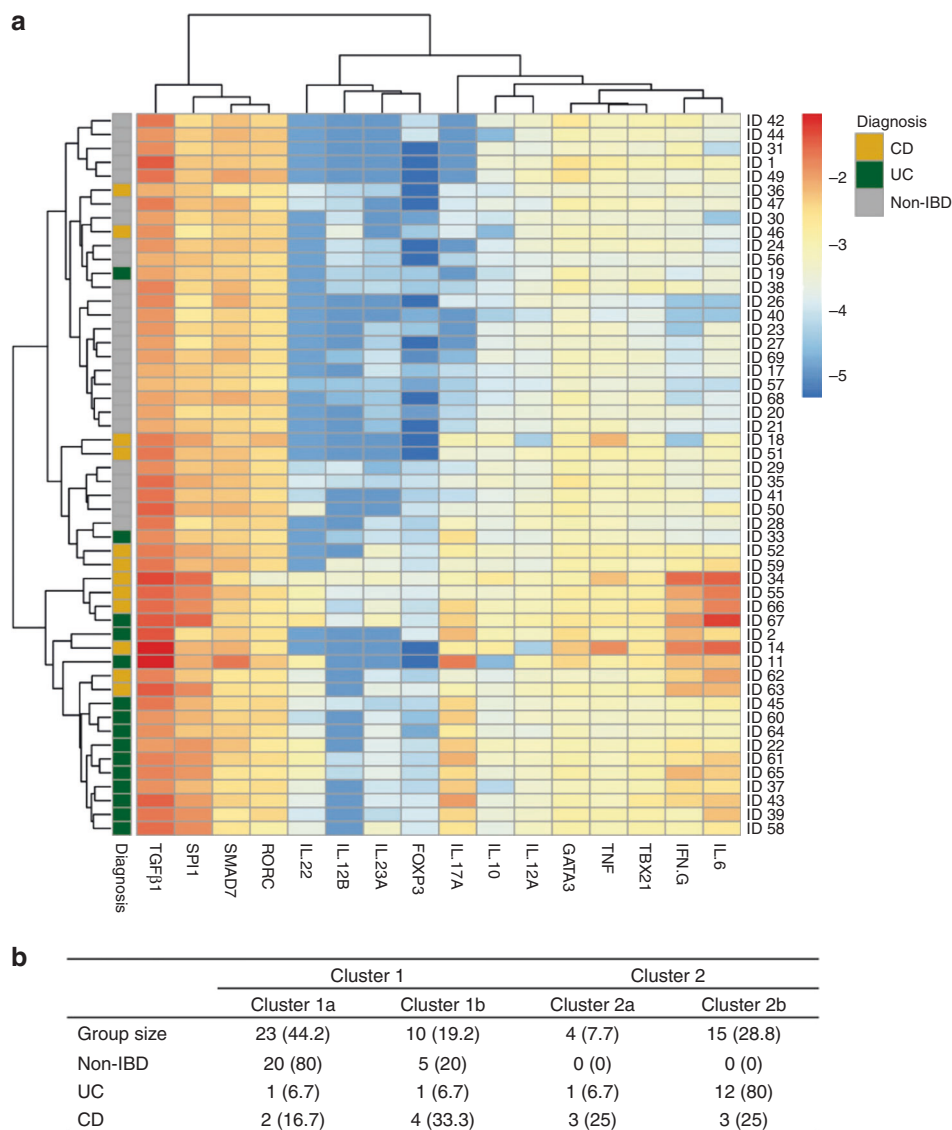


Fig. 4 Heatmap and gene expression cluster. **a** Heatmap. $2^{-\Delta C_q}$ values were coded by color: blue and red correspond to lower and higher levels of gene expression, respectively. ID numbers of the anonymized study participants are indicated on the right ordinate. Two dendrograms are integrated in the map, which enable grouping of patients (left) and genes (above). **b** Patient cluster. The patients were grouped into clusters based on the second division of the dendrogram shown on the left side of the heatmap. Values are shown as n (%)

Many findings obtained studying the pediatric cohort described here correlate with the results reported previously for adult IBD patients.^{7,30,31} The expression of genes encoding typical proinflammatory cytokines, such as TNF- α , IFN- γ , and IL-6, for example, was increased in both pediatric IBD groups concurrent with a less marked increase in the immunoregulatory cytokine, TGF- β 1. The elevated level of IL-23A mRNA found in tissues collected from both adult and pediatric patients supports the involvement of the IL-12/IL-23 axis in the pathogenesis of IBD.^{7,30,32} Indeed, in the absence of a considerable increase in IL-12 message expression (herein, IL-12B mRNA levels were often below the level of detection), it has been suggested that IL-23A is a major effector molecule in IBD.³³ Accordingly, ustekinumab and risankizumab (antibodies that bind and neutralize IL-12/IL-23 and IL-23A, respectively) have demonstrated efficacy in treating CD patients enrolled in clinical trials.³³ The results of our study were not altered significantly by excluding pre-treated patients in further analysis. Systemic treatment had negligible influence on the expression of targeted genes in the mucosa of our pediatric IBD cohort.

Clustering the patients based on patterns of gene expression revealed the possible distinction of disease entities, that is, UC. The ability to differentiate UC patients from non-IBD controls was unexpectedly sensitive given the limited number of patients enrolled in the study and genes included in analysis. This finding correlates with data published by Rosen et al.,¹⁶ in which the unsupervised hierarchical clustering of gene expression enabled differentiation of children with and without IBD. Clustering in their study was found especially among genes expressed by UC and non-IBD patients, although the extent of clustering was lower than reported herein. This difference was due, in part, to their additional analysis of T_h2-associated cytokine (i.e., IL-5 and IL-13) gene expression. Distinct patterns of gene expression in children stricken with CD and UC was also noted in a recent study.³⁴ The expression of over 16,000 genes in intestinal mucosal specimens was assessed, albeit no analysis of a non-IBD control group was conducted.³⁴ Any comparisons of these reports are limited by differences in methodology and the genes assessed. Nonetheless, they

validate the potential use of gene expression panel as a tool for diagnosing pediatric IBD.

A number of recent studies have discussed possible parameters that could be used to predict and monitor the success of IBD treatment. In this regard, current data correlating mucosal gene expression and treatment outcomes are scarce. Olsen et al.³⁵ reported an inverse correlation between pre-treatment levels of TNF- α mRNA expressed in colorectal biopsies and the clinical outcome of UC patients treated with infliximab, an antibody that binds and neutralizes both soluble and membrane-bound TNF- α ; treatment was least effective in patients who expressed very high TNF- α mRNA levels. Atreya and Neurath³⁶ reported instead that a better response to anti-TNF- α treatment occurred in patients who possessed a high percentage of cells that exhibited membrane-bound TNF- α , quantified by molecular imaging analysis. This latter finding correlates with the results of clinical trials that demonstrate treatment with antibody, for example, infliximab, which neutralizes both soluble and membrane-bound TNF- α , was highly effective by virtue of its capacity to induce apoptosis by TNF- α -expressing cells.⁷ Treatment with antibody capable of neutralizing soluble TNF- α only had no therapeutic value.

IL-23A plays a pivotal role in the pathogenesis of IBD.³³ Ustekinumab, an antibody that targets the p40 subunit composing IL-23A, as well as IL-12, offers an alternate approach to treating patients who are refractory to anti-TNF- α therapy. Ustekinumab treatment has demonstrated efficacy in clinical trials used to treat CD.³³ Tissue biopsies obtained from pediatric patients stricken with either UC or CD in the study described herein are characterized by the elevated expression of IL-23A mRNA relative to TNF- α message. Ustekinumab therapy provides a potential means of treating such children.

Interpretation of the results obtained in our study is limited by, first, the sample size. Regardless, the demographics of our study cohort and the results obtained were comparable to data present in the CEDATA-GPGE registry, confirming that our patients form a representative pediatric IBD cohort³⁷ [Supplemental Table S4 (online)]. Second, gene (mRNA) expression rather than protein production was quantified. Clearly, a number of factors in addition to mRNA synthesis affect the accumulation of proteins in the tissues.³⁸ Ethical constraints, however, limited the size of the tissue biopsy collected from children in the present study, eliminating the possibility of protein quantification. Third, a review of retrospective charts did not allow scoring of standardized disease activity, which is known to impact cytokine gene expression.^{19,39} Since the vast majority of patients enrolled in our study was newly diagnosed at the time of sample collection and disease activity was generally high, significant differences in gene expression seem unlikely due to variations in disease activity alone.

Taken together, our findings suggest a potential key role of IL-17A in the early course of IBD, albeit the nature of this role (beneficial or detrimental) remains to be clarified. We are among the first to describe the consistent, vigorous overexpression of IL-17A in pediatric UC, compared to CD or non-IBD, patients. Since the majority of patients in our IBD cohort was newly diagnosed and treatment-naïve, these data provide insight into the early mucosal response and the pathogenesis of IBD. Furthermore, analysis demonstrating the clustered expression of targeted genes supports the potential use of gene expression profiles as a diagnostic tool for pediatric IBD.

ACKNOWLEDGEMENTS

Results shown in this study are part of the doctoral thesis of M.A.B. We thank Dr. Stephen H. Gregory (Providence, RI, USA) for his help writing and editing this manuscript. M.A.B. received a personal grant through the Sibylle Kalkhof-Rose Foundation.

AUTHOR CONTRIBUTIONS

M.A.B. performed and analyzed experiments, interpreted the findings, and prepared the manuscript; R.L.K. supported acquisition of clinical data and critically revised the manuscript for important intellectual content; I.H. helped with the statistical analysis and graph editing; U.K. and V.B. performed the endoscopies and collected tissue samples; A.D. performed tissue preparation; C.U.M., B.G., and L.P. supported the experiments and gave critical input to the project; F.Z. was involved in the study design and in the interpretation of the immunological findings; S.G. designed the study, acquired funding, collected tissue samples, and critically revised the manuscript for important intellectual content.

ADDITIONAL INFORMATION

The online version of this article (<https://doi.org/10.1038/s41390-019-0486-5>) contains supplementary material, which is available to authorized users.

Competing interests: The authors declare no competing interests.

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

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