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The transcriptomic immune profiling across the different ileal layers highlights the relevant role of neutrophils and the active involvement of healthy mucosa in human Crohn's Disease

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ABSTRACT

Background and Aims: Crohn's disease (CD) is a chronic inflammatory disorder characterized by discontinuous and transmural inflammation of the gastrointestinal tract. While most studies have focused on mucosal immunity and individual immune cell subsets, little is known about the coordinated immune activity across the different ileal layers. This study aimed to characterize and compare, for the first time, the transcriptomic and immune landscape of the mucosa, submucosa, and serosa in diseased and adjacent healthy ileal tissue from CD patients.
Methods: Matched healthy and damaged ileal samples from 17 CD patients were dissected into three layers. Expression of 579 immune-related genes was quantified using the NanoString nCounter® GX Human Immunology V2 kit.
Results: Distinct transcriptional profiles were found between diseased and healthy tissues across all layers, with the mucosa showing the most pronounced dysregulation. Damaged mucosa exhibited upregulation of innate immunity-related genes, while neutrophil-associated transcripts and chemokines predominated in the serosa. Interestingly, healthy ileal tissue mirrored, layer by layer, the same distribution of innate (neutrophils) and adaptive (T and B) immune cells as diseased areas. A vertical immune gradient from mucosa to serosa was identified in both tissue types.
Conclusions: This study provides novel insights into the compartmentalized immune architecture of the ileum in CD. The findings highlight a potential role of healthy tissue and serosal neutrophils in CD pathogenesis and progression. Layer-resolved transcriptomic profiling could aid early diagnosis, reveal new biomarkers, and support the development of personalized therapeutic strategies targeting specific ileal compartments.

1. Introduction

Inflammatory bowel disease (IBD) encompasses a group of chronic conditions characterized by persistent inflammation of the gastrointestinal (GI) tract, including Crohn's disease (CD) [1,2]. Affecting over 6.8 million people worldwide, IBD represents a growing global health concern due to its increasing prevalence. Among IBD subtypes, CD is mainly prominent as it can affect any part of the GI tract. It is a complex disorder arising from an imbalanced immune response driven by a multifactorial interplay of environmental, genetic, and microbiome-related factors, with many of the underlying mechanisms yet to be fully elucidated. Current evidence suggest that CD develops in genetically susceptible individuals as a result of dysregulated immune responses, with altered expression of intestinal genes playing a key role in disease pathogenesis [3]. Several studies have documented that in CD patients, the mucosal immune system is over-activated, leading to an aberrant cytokines' production and sustained inflammation [4].

Even among patients with clinical remission, up to half still experience inflammation [5]. Approximately 50 % of the total risk for developing CD is attributed to genetics, with over 70 genes identified as contributing to its onset and progression [6].

CD is typically classified as a condition driven by T helper-1 (Th1)

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and −17 (Th17) cells, characterized by increased levels of interleukin-12 (IL-12), IL-23, interferon-gamma (IFN-γ), and IL-17 [7]. Even with effective therapies available, 30–50 % of patients either do not adequately respond, suffer from serious side effects, or eventually lose the treatment's effectiveness [8–10]. Nevertheless, although the right mechanisms behind the disease are not fully understood, it is widely accepted that the innate immune system plays a central role in beginning intestinal inflammation. Within the gut, the majority of pro-inflammatory immune cells are located in the mucosal layer, whereas the submucosa may contain fewer, primarily recruited, immune cells. In contrast, the inflammatory processes occurring in the serosa are still poorly characterized.

In a previous study, we documented that the amounts of cytokines involved in immune cell recruitment varies across the ileal wall, with distinct patterns observed between the submucosal and serosal compartments [11]. Currently, most research focuses on individual immune cells or specific subsets thereof, often through longitudinal studies. However, the immune system operates as a complex and dynamic network, in which immune cells interact extensively with one another and with a variety of molecular and environmental factors [12].

For these reasons, this study aimed to finely explore the transcriptomic signature of the intestinal mucosa, submucosa, and serosa in CD patients by analyzing gene expression patterns to better characterize the differences between damaged and surrounding healthy tissue, as well as to assess layer-specific alterations across the intestinal wall.

3. Results

3.1. Study population

Seventeen adult patients (eleven males and six females, with a mean age of 44 years, ranging from 21 to 74 years), diagnosed with ileal (16 patients) or ileocolic CD (1 patient), were enrolled (between January 2021 and September 2021) at 'Careggi University Hospital' (Florence, Italy). Among them, 47 % (8 patients) were diagnosed with stricturing disease, 35,3 % (6 patients) with stricturing-penetrating disease, and 17,7 % (3 patients) with penetrating CD. Of the enrolled patients, 52,9 % (9 patients) underwent their first surgery, while 47,1 % (8 patients) were enrolled during disease recurrence. Demographic and clinical data for each patient are summarized in Table 1.

3.2. Differentially expressed genes in damaged layers vs. healthy counterparts

The gene expression analysis of diseased *vs* surrounding healthy ileal layers identified distinct patterns of dysregulation. In sum, we identified 47 significantly upregulated genes ($\log_2(fc) \geq 1$, BH-$p < 0.05$), and 8 downregulated genes ($\log_2(fc) \leq -1$, BH-$p < 0.05$) in CDM compared to HM. CDSM and CDS exhibited less dramatic gene expression changes, with 12 and 2 upregulated genes, respectively $\log_2(fc) \leq -1$, BH-$p < 0.05$)(Fig. 1). Among these, comparing to the healthy counterparts, 17 genes (FCGR2A, TAGAP, ARG2, THY1, LILRA5, SOCS3, FCGR3A/B, LY96, FCGR2A/C, CD24, IDO1, PLAU, IL1B, CXCL10, IL1R2, MUC1, IL8) showed a strong differential expression ($\log_2(fc) \geq 2$ or ≤ -2, BH-$p < 0.05$) in CDM and 3 (CD79A, CXCL9, CD27) in CDSM.

In detail, CDM samples exhibited the highest number of upregulated genes associated with the inflammatory response. Notably, IDO1 ($\log_2(fc)=2.75$, BH-$p = 0.004$), LY96 ($\log_2(fc)=3.23$, BH-$p = 0.003$), SOCS3 ($\log_2(fc)=2.54$, BH-$p = 0.003$) and MUC1 ($\log_2(fc)=2.26$, BH-$p = 0.023$) were strongly upregulated. Additionally, CDM samples showed pronounced upregulation of genes associated with cytokine signaling, including IL1β ($\log_2(fc)=3.37$, BH-$p = 0.008$) and IL1R2 ($\log_2(fc)=2.23$, BH-$p = 0.023$).

Interestingly, no cytokine or cytokine receptor-encoding genes were detected in the deeper layers, except for CD27 in CDSM ($\log_2(fc)=2.32$, BH-$p = 0.034$) (Table 2). Additionally, in CDM, multiple chemokines

Table 1
Demographic and clinical characteristics of the enrolled patients with Crohn's disease (CD).

No.	Sex[a]	CD site[b]	Disease behavior[c]	CDAI	Surgery	Age at 1st surgery	Age at recurrence surgery	Smoking status[d]	Familiarity	Fistulae	Abscesses	History of perianal disease	Active perianal disease	Discontinued CD therapies (within 4 weeks prior to surgery)
1	M	L1	B2	>250	1st surgery	21	-	Yes	No	No	No	No	No	MS
2	M	L1	B2-B3	<300	1st Surgery	74	-	Yes	Ns	Yes	Yes	Ns	No	MS, CT
3	M	L1	B2-B3	>300	Recurrence	36	40	No	No	Yes	Yes	No	No	MS, CT, ADA
4	M	L1	B2	>250	Recurrence	67	67	No	No	No	No	Yes	No	CT
5	F	L1	B2-B3	>300	1st surgery	49	-	Yes	Ns	Yes	No	No	No	MS
6	F	L1-L2	B2	>250	1st surgery	47	-	No	No	No	No	No	No	CT, ADA
7	F	L1	B3	>300	Recurrence	28	33	No	No	Yes	Yes	No	No	None
8	M	L1	B2-B3	>300	Recurrence	16	37	Yes	No	Yes	No	Yes	No	UM, MT
9	M	L1	B3	>300	Recurrence	30	31	No	Ns	Yes	Yes	No	No	None
10	M	L1	B2	>250	1st surgery	24	-	No	Ns	No	No	No	No	MS
11	M	L1	B2	>250	1st surgery	26	-	No	Ns	No	No	No	No	NONE
12	M	L1	B2	>250	Recurrence	30	43	No	Ns	No	No	No	No	MS
13	F	L1	B3	>300	1st surgery	27	-	No	No	Yes	No	No	No	MS
14	M	L1	B2	>250	Recurrence	57	63	Yes	Ns	No	No	No	No	MS, CT
15	F	L1	B2-B3	>300	1st surgery	50	-	No	No	Yes	Yes	No	No	MS
16	M	L1	B2-B3	>300	Recurrence	26	36, 46	No	No	Yes	Yes	No	No	None
17	F	L1	B2	Ns	1st surgery	54	-	No	No	Ns	Ns	Ns	Ns	MT

Abbreviations: ADA, Adalimumab; CD, Crohn's disease; CDAI, Crohn's Disease Activity Index; CT, Corticosteroids; MS, Mesalamine; MT, Metronidazole; Ns, not specified; UK, Ustekinumab.

[a] Sex: M, male, F, female.
[b] CD site: L1, terminal ileum, L2, ileum colon.
[c] Disease behavior: B2, Stricturing; B3, Penetrating.
[d] Smoking status: Yes, current smoker; No, non-smoker.

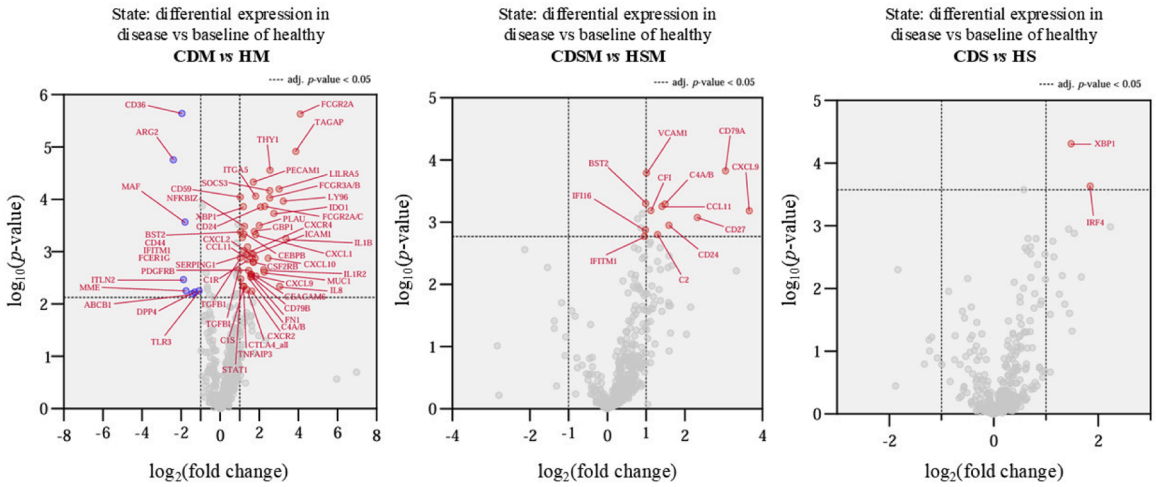

Fig. 1. Volcano plots showing the differentially expressed genes in damaged tissue *vs.* healthy tissue. Red dots represent genes exhibiting significant upregulation, defined as $\log_2$(fold change) ≥ 1 and BH-$p< 0.05$; purple dots indicate genes with significant downregulation, defined as $\log_2$(fold change) ≤ -1 and BH-$p< 0.05$. Genes not meeting these criteria are depicted in gray. CDM, damaged mucosa; CDSM, damaged submucosa; CDS, damaged serosa; HM, healthy mucosa; HSM, healthy submucosa; HS, healthy serosa.

Table 2
Differentially expressed genes in CDM *vs.* HM. For each gene with $\log_2$(fold change) $\geq +1$ (overexpressed) or ≤ -1 (downregulated), $\log_2$(fold change) is presented, along with an adjusted *p*-value. [a]BH, Benjamini-Hochberg.

Gene	$\log_2$(fold change)	BH.*p*-value[a]	Gene	$\log_2$(fold change)	BH.*p*-value[a]
CD36	−1.96	0.000475	IL1B	3.37	0.00841
FCGR2A	4.1	0.000475	IFITM1	1.4	0.0113
TAGAP	3.88	0.00165	FCER1G	1.22	0.0128
ARG2	−2.39	0.00179	CXCR4	1.58	0.0138
THY1	2.55	0.00225	ICAM1	1.69	0.0138
PECAM1	1.7	0.00317	SERPING1	1.35	0.0138
LILRA5	3.02	0.00346	TGFB1	1.03	0.015
SOCS3	2.54	0.00346	CXCL10	2.46	0.015
ITGA5	1.82	0.00349	CXCL2	1.79	0.015
CD59	1.03	0.00349	C1R	1.39	0.0155
FCGR3A/B	2.54	0.00349	CSF2RB	1.69	0.0167
LY96	3.23	0.00354	CCL11	1.71	0.0167
FCGR2A/C	2.29	0.00354	IL1R2	2.23	0.0226
XBP1	1.19	0.00354	PDGFRB	1.46	0.0227
CD24	2.07	0.00354	MUC1	2.26	0.0232
IDO1	2.75	0.00446	FN1	1.56	0.0235
MAF	−1.79	0.00616	CEACAM6	1.59	0.0235
PLAU	2	0.00635	CD79B	1.63	0.0241
NFKBIZ	1.25	0.00635	CXCL9	1.84	0.0241
GBP1	1.76	0.00741	C4A/B	1.57	0.0244
BST2	1.05	0.00741	STAT1	1.06	0.0258
CEBPB	1.22	0.00767	ITLN2	−1.88	0.0263
CXCL1	1.82	0.00767	TNFAIP3	1.2	0.0335
CD44	1.14	0.00835	C1S	1.22	0.0335
TLR3	−1.07	0.037	IL8	3.05	0.0335
CXCR2	1.62	0.037	CTLA4_all	1.38	0.037
DPP4	−1.3	0.0385	MME	−1.74	0.037
ABCB1	−1.42	0.0397			

were upregulated, including CXCL10 ($\log_2$(fc)= 2.46; BH.*p*= 0.015) and IL-8 ($\log_2$(fc)= 3.05; BH.*p* = 0.033). Conversely, in CDSM, only two chemokines were significantly upregulated, among which CXCL9 showed a strong expression ($\log_2$(fc)= 3.66; BH.*p* = 0.034) (Table 2 and Table 3).

Notably, CDM was the only tissue layer exhibiting a significant downregulation of immune-related genes, specifically those involved in the negative regulation of inflammation, including CD36 ($\log_2$(fc)=−1.96, BH-*p* = 0.0005) and ARG2 ($\log_2$(fc)=−2.39, BH-*p* = 0.002).

In addition, CDM showed a strong upregulation of genes encoding

Table 3
Differentially expressed genes in CDSM *vs.* HSM. For each gene with $\log_2$(fold change) $\geq +1$ (overexpressed) or ≤ -1 (downregulated), $\log_2$(fold change) is presented, along with an adjusted *p*-value. [a]BH, Benjamini-Hochberg.

Gene	$\log_2$(fold change)	BH.*p*.value[a]
CD79A	3.05	0.0311
VCAM1	1.01	0.0311
BST2	1	0.0341
C4A/B	1.49	0.0341
CCL11	1.41	0.0341
CFI	1.12	0.0341
CXCL9	3.66	0.0341
CD27	2.32	0.0341
CD24	1.59	0.0394
IFI16	1	0.0396
C2	1.3	0.0433
IFITM1	1	0.0434

cell-surface receptors involved in innate immune responses, such as FCGR2A ($\log_2$(fc)=4.1, BH-*p* = 0.0005), FCGR3A/B ($\log_2$(fc)=2.54, BH-*p* = 0.003), FCGR2A/C ($\log_2$(fc)=2.29, BH-*p* = 0.003), LILRA5 ($\log_2$(fc)=3.02, BH-*p* = 0.003) and CD24 ($\log_2$(fc)=2.07, BH-*p* = 0.003) (Table 2), while genes involved in the complement system were upregulated in CDSM, as shown in Table 3.

Finally, several genes involved in T cell- and B cell-mediated responses showed a differential expression across different tissue layers (Tables 2–4). Notably, our samples showed a strong upregulation of TAGAP ($\log_2$(fc)=3.88, BH-*p* = 0.002) and THY1 ($\log_2$(fc)=2.55, BH-*p* = 0.002) in CDM while CD79A ($\log_2$(fc)=3.05, BH-*p* = 0.031) was upregulated in CDSM.

Table 4
Differentially expressed genes in CDS *vs.* HS. For each gene with $\log_2$(fold change) $\geq +1$ (overexpressed) or ≤ -1 (downregulated), $\log_2$(fold change) is presented, along with an adjusted *p*-value.

Gene	$\log_2$(fold change)	BH.*p*.value[a]
XBP1	1.48	0.0173
IRF4	1.85	0.0313

[a] BH, Benjamini-Hochberg.

3.3. Immune-pathway scores were differentially regulated in damaged vs. healthy layers

Nanostring pathway score analysis revealed a trend of upregulation across several pathway signatures in all three diseased ileal layers compared to their healthy counterparts (Fig. 2). Notably, pathway scores for NF-κB signaling, cytokine and chemokine signaling underscored the involvement of all three damaged ileal layers in the inflammatory response (Fig. 2b). Furthermore, pathways with the highest scores were predominantly associated with innate immunity and lymphocyte activation in all three tissue layers, and host-pathogen interactions in CDSM and CDS (Fig. 2b).

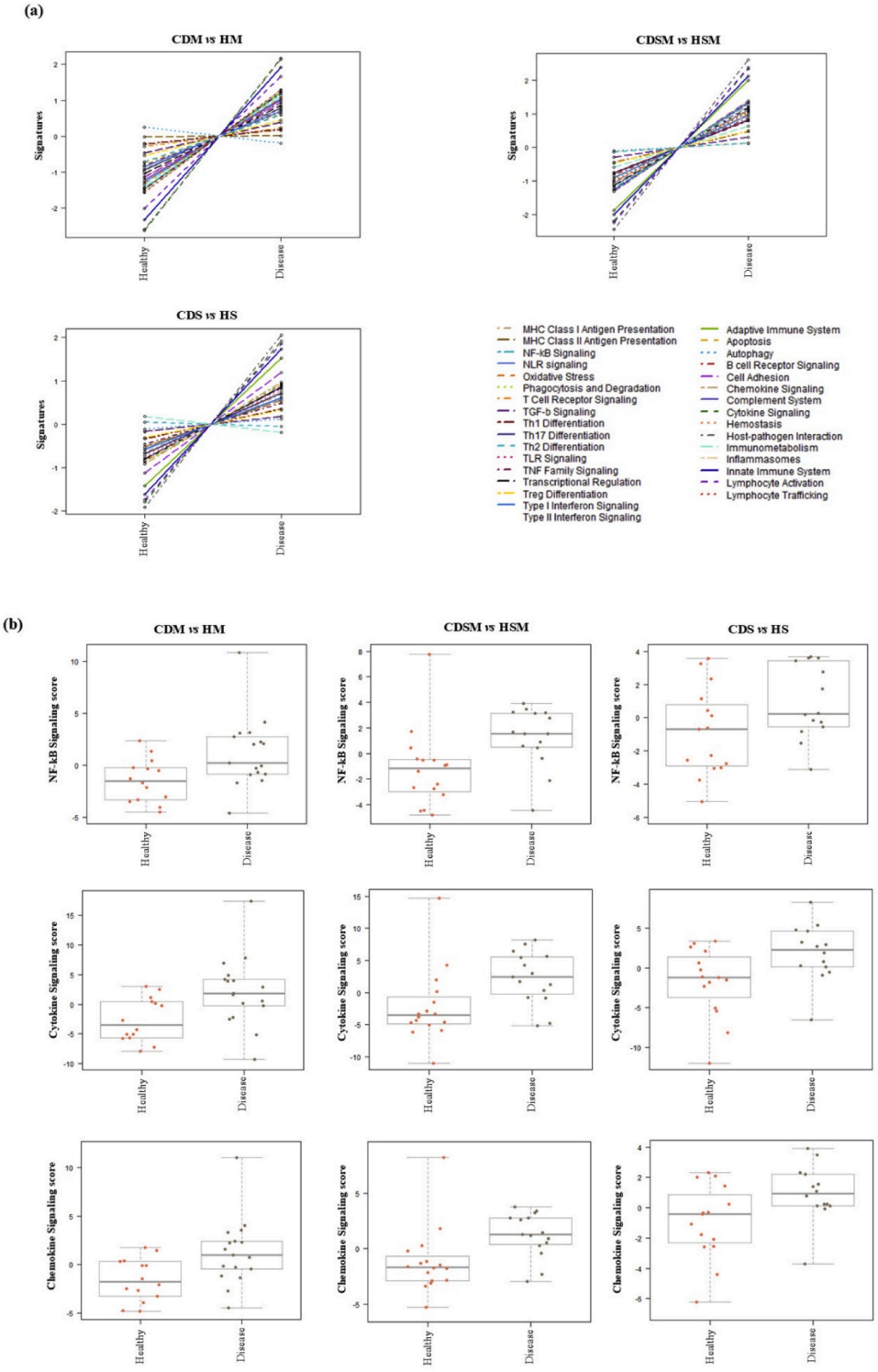

Fig. 2. (a) Trend plots illustrating broad upregulation in immune pathway scores in damaged tissues; (b) Box-plots depicting pathway score signatures involved in inflammation, highlighting a marked dysregulation in NF-kB, cytokine and chemokine pathway activity between damaged and healthy tissues. CDM, damaged mucosa; CDSM, damaged submucosa; CDS, damaged serosa; HM, healthy mucosa; HSM, healthy submucosa; HS, healthy serosa.

3.4. Cell-type profiling

The overall abundance of most cell type profiles was elevated across the wall of the diseased ileum, as depicted in the trend plots in Fig. 3. In addition, using the nCounter® advanced analysis module, we assessed the abundance of immune cell populations characterized by cell type-specific expression patterns. The raw cell type measurements revealed an increase in neutrophils and immune cells ($CD45^+$) in CDM; T cell levels did not show a clear decrease in median values between healthy and diseased mucosa, but CDM exhibited greater variability and included notably lower individual scores. In contrast, CDSM exhibited the highest scores for $CD45^+$ cells and T-cells, with similar trends observed in CDS (Fig. 3).

3.5. Transmural gene expression pattern and cell-types in damaged and healthy ileum

The vertical analysis of differentially expressed genes across the tissue layers of both healthy and diseased ileum revealed distinct gene expression profiles along the intestinal wall. Using pairwise comparisons, we identified 28 genes that were differentially expressed in both CDSM and CDS compared to CDM ($\log_2(fc) \geq 1$ or ≤ -1 and BH.$p < 0.05$) (Fig. 4a) and 33 genes differentially expressed in both HSM and HS compared to HM (Fig. 4d), allowing us to define a gradient of gene expression from the mucosa to the serosa for both healthy and diseased ileum. Additionally, 9 genes were uniquely differentially expressed in CDSM *vs*. CDM ($\log_2(fc) \geq 1$ or ≤ -1 and BH.$p < 0.05$) (Fig. 4b), and 43 genes in CDS *vs*. CDM ($\log_2(fc) \geq 1$ or ≤ -1 and BH.$p < 0.05$) (Fig. 4c). Notably, no significant differences were observed in CDS *vs.* baseline of CDSM. For what concerns the healthy tissues, 1 gene was uniquely differentially expressed in HSM *vs*. HM ($\log_2(fc) \geq 1$ or ≤ -1 and BH.$p < 0.05$) (Fig. 4e) and 78 genes in HS *vs*. HM ($\log_2(fc) \geq 1$ or ≤ -1 and BH. $p < 0.05$). Similarly, to the diseased ileum, no significant differences were observed in HS *vs*. baseline of HSM.

To further visualize these gradients, we constructed a heatmap (Fig. 4) in which the mucosa was set as the baseline, assigning a $\log_2$(fold change) value of zero to all genes. The subsequent rows represented the submucosa and serosa, displaying their respective $\log_2$(fold change) values relative to the baseline (CDM). This approach allowed us to clearly observe the progression of gene expression changes through the intestinal layers and capture the transmural heterogeneity in healthy and diseased tissue, thus helping capture the dynamics of immune responses.

Starting from these different genetic expression patterns and cell type profiling results, we documented a significant gradient of immune cell distribution across the CD and healthy tissues. Specifically, the analysis indicated a putative increased abundance of $CD45^+$ immune cells near the luminal surface in both damaged and healthy ileum (CDM and HM), with a progressive decline in the deeper submucosa e serosa. In fact, further analyses of specific immune cell populations within the diseased tissues showed a decreasing trend in T and B cells, and cytotoxic cells from CDM to CDS, with a similar trend for T cells and cytotoxic cells in the healthy counterparts. Notably, neutrophils exhibited an opposite pattern, with higher scores in both diseased and healthy serosa compared to healthy and diseased mucosa and submucosa (Fig. 5).

4. Discussion

Our results provide a comprehensive view of the dynamics of gene expression across the three distinct layers of the intestinal wall in CD patients. Unlike previous similar research that focused mainly on the lamina propria, our study stands out as the first to innovatively investigate differentially expressed immune-related genes in the mucosa, submucosa, and serosa, moving beyond the traditional focus only on ileal mucosa, and considering the same approach also in adjacent healthy tissues. Thus, predicting pathway scores and cell-type profiles in analyzed tissues, we documented how the typical discontinuity of the intestinal inflammation in human CD implies distinct underlying gene expression profiles between visibly diseased areas and contiguous healthy tissues in the same patients.

A weakened intestinal barrier plays a crucial role in this process [3]. It is widely believed that inflammatory lesions first develop in the intestinal mucosa and that the progression of transmural inflammation allows the involvement of the submucosa, muscularis, and serosa in CD patients [13]. Specifically, IBD flare-ups are characterized by neutrophil transepithelial migration in the gut. Disordered architecture, neutrophil

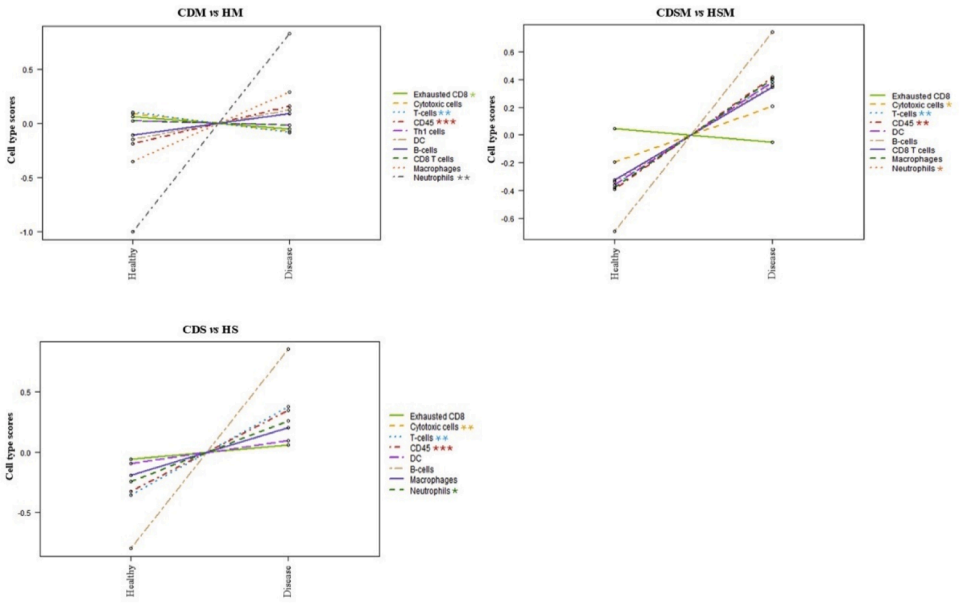

Fig. 3. Cell Type Profiling results suggesting increased immune cell infiltration across all three layers of the diseased ileum. The abundance of cell populations was assessed based on the expression of specific marker genes associated with each cell type. The trend plots depict the raw scores for each cell type, providing insights into the putative abundance of immune cells across the different layers of the disease-affected ileum. CDM, diseased mucosa; CDSM, diseased submucosa; CDS, diseased serosa; HM, healthy mucosa; HSM, healthy submucosa; HS, healthy serosa. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

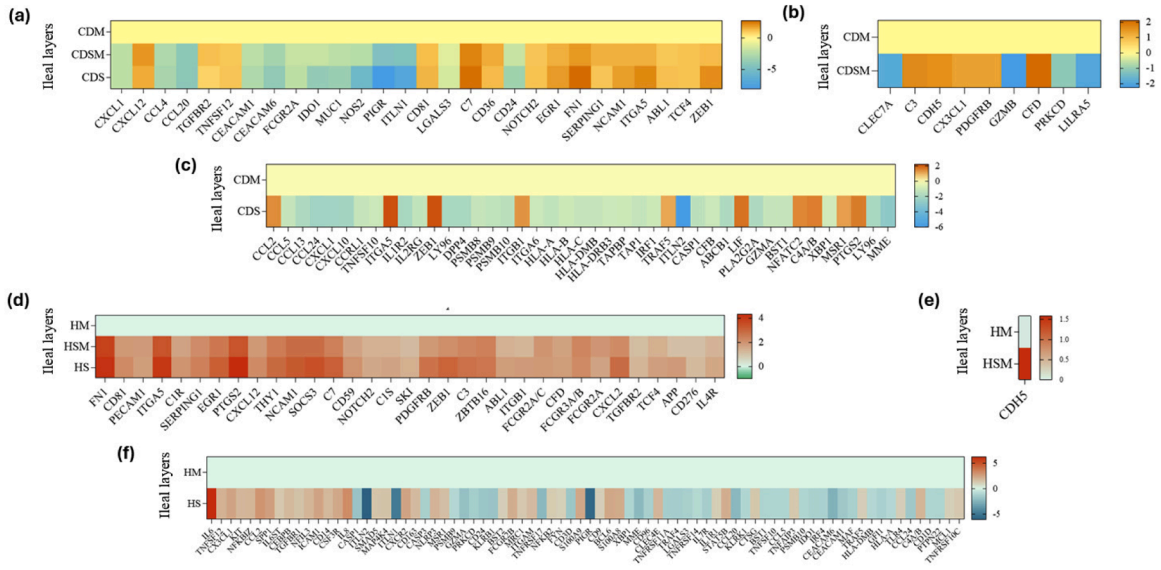

Fig. 4. Heatmaps showing the gradient of differentially expressed genes across the layers of diseased (a-c) and healthy (d-f) ileum with $\log_2(fc) \geq 1$ or ≤ -1 and BH. $p < 0.05$. (a, d) Heatmaps (a) and (d) represent the overall gene expression gradient across the diseased and healthy mucosa, submucosa, and serosa, with the mucosa set as baseline. Submucosa (CDSM, HSM) and serosa (CDS, HS) rows display their respective $\log_2(fc)$ values relative to the mucosa (CDM, HM), highlighting the progression of gene expression changes across the intestinal wall. (b, e) Heatmaps (b) and (e) show the uniquely differentially expressed genes in submucosa compared to mucosa, emphasizing gene expression alterations within the intermediate layer, not reported in the serosa. (c, f) Heatmaps (c) and (f) depict the uniquely differentially expressed genes in the serosa compared to the mucosa, illustrating the gene expression changes specific of the outermost layer. CDM, damaged mucosa; CDSM, damaged submucosa; CDS, damaged serosa; HM, healthy mucosa; HSM, healthy submucosa; HS, healthy serosa.

transepithelial migration with crypt abscesses, and extensive mucosal ulcers linked to neutrophil infiltration are typical histological characteristics of intestinal biopsies [14,15].

Our findings confirm the CD complexity, driven by multiple cell types contributing to the chronic inflammatory milieu, [16] mainly in the lamina propria [17]. Among these, neutrophils are known to be a major source of pro-inflammatory cytokines and chemokines and play a critical role in the inflammation endurance [18,16]. Our gene expression analysis revealed increased cell-type scores characterized by a widespread presence of neutrophils when comparing damaged and healthy tissues, different from the transmural scores obtained in the vertical analysis, which were higher within the diseased serosa compared to the upper CD layers. These findings suggest a prominent role for neutrophils in driving transmural inflammation, consistent with the known pathological hallmark of polymorphonuclear neutrophils infiltration in IBD [19]. Neutrophils possess strong antimicrobial activity and have been implicated in pathological intestinal inflammation through the production of reactive oxygen species by the neutrophil NADPH oxidase [20]. Moreover, these cells are highly responsive to IL-8, [21] a chemokine for which we have documented increased expression levels in the CD mucosa compared to healthy mucosa, as well as elevated protein levels in patients with active CD, as we have previously reported [11]. However, the neutrophils' recruitment to a specific tissue site through chemokines alone, without their simultaneous activation, is not enough to induce tissue damage, as demonstrated in a transgenic mouse model with doxycycline-induced human IL-8 expression in the ileal and cecal epithelium [22]. In addition, our data showed increased expression levels of the chemokines' genes CXCL1 and CXCL2, alongside increased levels of CXCR2-mRNA. These chemokines are secreted by monocytes and macrophages and are chemotactic for polymorphonuclear leukocytes. When CXCL1 and CXCL2 bind to CXCR2, they activate chemokine pathways that are primarily responsible for the recruitment and, notably, activation of neutrophils in response to inflammatory stimuli [23].

In contrast to the well-established proinflammatory role of neutrophils, Kuhl A *et al.* investigated the effects of neutrophils depletion in a mouse model of chronic T cell-mediated colitis. In this model, disease is induced by transferring CD45RBhigh T cells (without regulatory T cells) into T cell-deficient RAG-1 knockout mice. Unlike DSS- or hapten-induced colitis models, this approach more closely resembles human IBD, as it involves T cell-mediated responses to gut microbiota-derived antigens [24]. Surprisingly, neutrophil depletion, during the T cell transfer, exacerbated colitis and led to earlier mortality, [24] highlighting a more complex role for these immune cells in intestinal inflammation.

In other words, these findings challenge the outdated view of neutrophils as solely proinflammatory and destructive in IBD, suggesting that they may also play a protective role [25] and raising the question of whether their presence in chronic disease mirrors a pathogenic mechanism or a compensatory response.

Notably, in support of the potential protective role of neutrophils, for the first time we have documented a similar increase (comparing healthy mucosa and submucosa) of these relevant cells also in healthy serosa. In addition, this finding suggests that the immunity cells located in healthy ileum could play a relevant role in CD development.

In fact, beyond neutrophils, the transmural inflammatory infiltrates in CD patients are characterized by effector T cells and many other leukocyte populations, which promote IBD [26,27] and our data suggest that T cells are likely more abundant in the diseased submucosa and serosa compared to the adjacent healthy ileum, which exhibited higher cell-type scores within the mucosa.

But in the meantime, we observed an increased abundance of $CD45^+$ immune cells near the luminal surface in both diseased and healthy ileum (CDM and HM), with a progressive decline in the deeper submucosa e serosa. In fact, further analysis of specific immune cell populations within the damaged tissues showed a decreasing trend in T and B cells, and cytotoxic cells from CDM to CDS, with a similar trend for T cells and cytotoxic cells in the healthy counterparts. Interestingly, the increased scores in the healthy mucosa may mirror the presence of exhausted $CD8^+$ *T*-cells, whose typical markers were elevated in this tissue layer. Usually, the T cell exhaustion occurs when T cells are persistently activated by antigens, leading to a decline in their effector functions (such as cytokine secretion) and an increase in the expression of inhibitory receptors [28]. The persistence of less responsive,

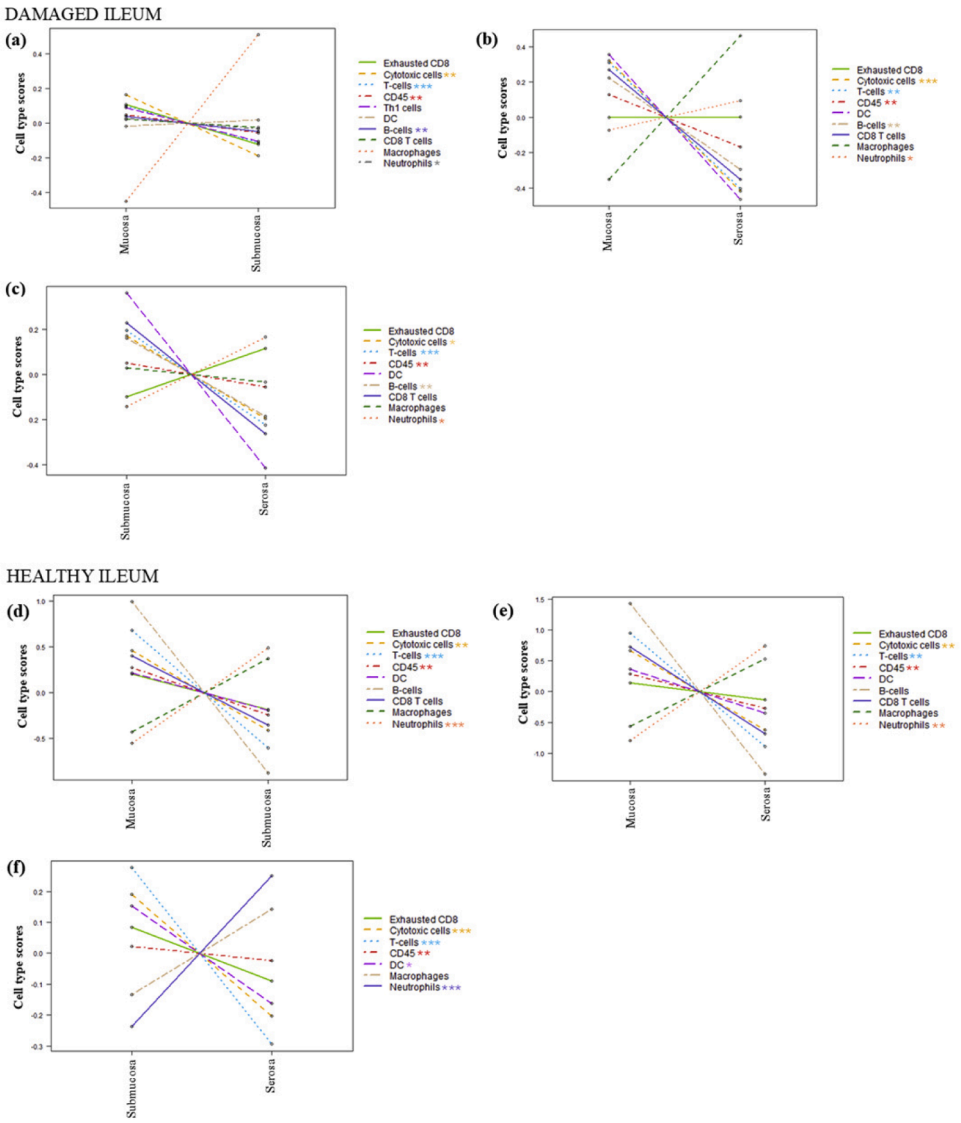

Fig. 5. Cell Type Profiling of pairwise comparisons across the different layers of damaged (a, b, c) and healthy (e, f, g) ileum. The putative abundance of cell populations was assessed based on the expression of specific marker genes associated with each cell type. (a-f) Trend plots depicting the raw scores for each cell type. * $p< 0.05$, ** $p< 0.001$, *** $p< 0.001$.

exhausted T cells during chronic antigen exposure may help partially limit immunopathology, as demonstrated by McKinney F *et al.* who associated exhausted $CD8^+$ expression patterns with a less severe disease progression and a more favorable prognosis [29]. The putative abundance of exhausted $CD8^+$ *T* cells observed in the healthy mucosa could indicate a homeostatic role, as these cells are known to be less reactive to microbial luminal content [30]. In other words, we can suggest a potential protective mechanism in maintaining mucosal integrity in the healthy ileum with a critical homeostatic role of the exhausted cytotoxic T cells.

While dysregulated T cell responses have long been documented in the CD pathogenesis, recent research has highlighted the involvement of both immunity branches, innate and adaptive, with growing evidence of altered function of innate receptors, [31] such as Fcγ receptors (FcγRs). Even though FcγRs signaling has been extensively studied in ulcerative colitis, with links to IL-1β production and type-17 immunity [32], its role in CD remains not fully explored. Nevertheless, our results emphasize the potential of such signaling pathways in CD pathogenesis. In fact, a key finding of our study was the strong upregulation of three activating FcγRs (FCGR2A, FCGR3A/B, FCGR2A/C) in the diseased mucosa. These genes exhibited the highest scores within our panel, and such results suggests enhanced phagocytic activity in the visibly damaged areas of the ileum, where IL-1β expression was also significantly elevated, supporting our previous protein-level findings [11]. Given the known increase of IgG-bound bacteria [33] and B cells within tissue immune infiltrates in IBDs [34], the strong upregulation observed for FcγRs suggests a role for phagocytic responses in mucosal inflammation, which may increase the downstream inflammatory cascade in the inflamed gut areas, a mechanism that warrants further investigations in CD. Finally, the heightened expression of the regulatory leukocyte receptor LILRA5 in the diseased mucosa, alongside CXCR2, CXCL1, IL-1β and IL-8, corroborates findings by Dheer et al., who described a similar phagocytic profile within the lamina propria of human IBD biopsies [35].

FcγR signaling is crucial for the production of several chemokines, including IL-8 and CXCR3-related chemokines such as CXCL9 and CXCL10 [36]. In our analysis, CXCL9 was upregulated in both the diseased mucosa and submucosa, showing no significant vertical gradient across the diseased ileal layers. In contrast, CXCL10 was specifically upregulated in the mucosal diseased ileum. These findings are consistent with those reported by Jones *et al.*, who observed increased mRNA levels of CXCR3 ligands in ileal CD patients [37] Notably, these

chemokines have been associated with CD recurrence [38] and were described by Singh et al. as IBD hallmarks [39].

Moreover, CXCR3-related chemokines are known to promote the activation of human effector T cells [37] and mediate neutrophils' recruitment to inflammatory sites in mouse colitis models [40]. Isles *et al.* further demonstrated that, after recruitment, neutrophils retention at inflammatory sites in Zebrafish involves additional chemokines such as CXCL12 [41], which is expressed and upregulated in human intestinal epithelial cells (IECs) of IBD patients [42]. While CXCL12 overexpression has previously been reported in the mucosa of CD patients, [42] a key finding of our study was the increased mRNA expression in the diseased submucosa and serosa. This was accompanied by cell-type scores indicating a putative higher neutrophils' abundance in the diseased serosa compared to the upper layers. These results suggest that CXCL12 may contribute to transmural inflammation and neutrophil persistence in CD. The localized expression CXCL12 pattern supports the presence of an organized immune response, driven by a complex chemotactic gradient extending beyond the mucosa into deeper layers, influencing the positioning and activity of granulocyte across the ileal wall.

Further analyzing genes implicated in innate immunity, our results underscore the pivotal role of other genes involved in innate-immune receptors signaling in the epithelial layer of the diseased ileum. The significant upregulation of LY96, encoding MD-2, a key component in the response to lipopolysaccharides (LPS), suggests heightened sensitivity to LPS in visibly inflamed areas. This result corroborates previous findings that IECs from IBD patients show increased LPS responsiveness through MD-2 upregulation, [43] compared to healthy IECs being physiologically poorly responsive to LPS [43,44]. MUC1 is another gene normally expressed at low levels in IECs but playing a crucial role in maintaining the integrity of intestinal barrier [45]. In the diseased mucosa, we observed a significant upregulation of this gene, further corroborating findings by Hashash et al. who associated its expression with endoscopic recurrence and postoperative outcomes in CD patients [46]. Similarly, PLAU and IDO1, both markedly upregulated in our samples, have well-established roles in UC and are linked to disease severity [47,48],. Alongside these genes, TAGAP was strongly expressed in the diseased mucosa and has been reported to vary with disease severity and location, showing the highest levels in cases of severe colonic CD [49]. SOCS3, a key regulator of cytokine-mediated STAT3 signaling and strongly associated with disease activity in UC [50–52], showed a strong upregulation in the damaged mucosa, while CD24, known to be differentially expressed between diseased and healthy tissues in IBDs, was notably upregulated in the diseased ileum [53].

Collectively, the innovative aspect of our findings lies in the potential of these genes, firstly studied in UC, to form a predictive panel for assessing disease activity, endoscopic recurrence, and surgical outcomes, lastly aiding in the prevention of complications and relapse in active CD. Furthermore, our layer-specific analysis of the ileum revealed that several genes previously linked to overall CD severity display distinct expression profiles across individual tissue layers. These findings suggest that future diagnostic tools could leverage such differences to develop more refined, layer-targeted panels for the early prediction of clinical and surgical recurrence.

Beyond the immunological disruptions observed, our data highlight the molecular complexity of the CD microenvironment, indicating alterations in metabolic pathways within the diseased ileum. Our findings point to an underlying immune-metabolic imbalance that may exacerbate inflammation and contribute to the differences observed between diseased and healthy ileal areas [54]. Notably, we identified a strong downregulation of the Arginase-2 encoding gene (ARG2), which is known to play a protective role against oxidative stress in UC [55]. While the role of arginine metabolism in IBD remains debated, our results contrast with previous reports of elevated Arginase-2 levels during active UC, [56] instead aligning with emerging evidence of impaired arginase pathways in IBDs [57]. Additionally, we detected a downregulation of CD36 in the diseased mucosa - a gene encoding a glycoprotein critical for the uptake of dietary lipids, including long-chain fatty acids (LCFA) and cholesterol [58]. These data suggest that disrupted lipid metabolism may contribute to chronic gut inflammation and epithelial barrier dysfunction in CD [59].

Surely our study shows some limits, such as the restricted number of enroll patients and some speculative conclusions, but summarizing, our data provide novel insights into pathogenesis of human CD by comprehensively analyzing gene expression across the distinct ileum layers, revealing unique expression patterns that may explain the characteristic patchy and discontinuous inflammation observed in CD patients. While inflammation and immune activation were evident in all diseased layers, consistent with the transmural nature of the Crohn disease, the mucosa and submucosa emerged as the primary compartments driving the inflammatory response.

In addition, and remarkably, we documented for the first time that the healthy ileal parts of the CD patients mirror, layer by layer, the same functional distribution of innate (neutrophils) and adaptative (T and B cells) immune cells in the ileum, highlighting a potential involvement of the healthy gut in the CD pathogenesis. To better clarify the role of this currently neglected aspect, could be crucial to fine evaluate the role of healthy ileum part in clinical progression such as the comparison between first surgery *vs.* surgical relapse patients, as suggested by our previous studies [60].

Moreover, it would be a priority to better investigate the functional characteristics of neutrophils, which we have shown to be highly represented in the serosa ileum of both parts (diseased and healthy) to rightly decipher their role, which in light of our data, appears controversial in the CD development.

By emphasizing the layered architecture of the intestinal wall and its compartmentalized inflammatory responses, our study advances our understanding of human CD but also underscores opportunities for developing more precise diagnostic tools and targeted therapies to tackle the disease's heterogeneity, thereby enhancing patient's quality of life.

In conclusion, we provided a well fine picture of the dynamics of gene expression across the different ileum layers in CD patients, innovatively exploring the differentially expressed immune-related genes in the mucosa, submucosa, and serosa, moving beyond the traditional focus only on ileal mucosa. We remarked the different distribution and function of immune cells in the various ileum layers highlighting the potentially relevant role of healthy mucosa and neutrophils in the modulation of human CD development.

2. Materials and methods

2.1. Study design

Seventeen patients with ileal or ileocolic CD diagnosis were enrolled between January 2021 and September 2021 at 'Careggi University Hospital' (Florence, Italy). The CD diagnosis was confirmed through clinical evaluations, endoscopic procedures, and histopathological analysis. To be eligible, patients had to meet the following criteria: (i) a confirmed CD diagnosis for at least three months following the Montréal classification; (ii) ileal or ileocolic localization; (iii) an age range between 20 and 75 years. Patients were excluded if they: (i) had recent (< 3 months prior to surgery) bacterial or parasitic GI infections; (ii) had traveled to exotic locations in the last five years. Notably, all the enrolled patients discontinued the use of antibiotics, aminosalicylates, immunosuppressants, biologic therapies, or steroids for a minimum of four weeks prior to surgery. The study protocol received approval from the local Ethics Committee (study no 2016.0842), and all participants provided written informed consent before inclusion.

2.2. Tissue sample preparation

Tissue samples from both disease-affected and healthy ileal regions were obtained from each patient as previously described [11]. Briefly, the ileocolic resection (ICR) was carefully divided into three layers (mucosa, submucosa, muscolaris/serosa) using a cold-blade scalpel under 4.5x magnification, performed by the same surgeon (G.F.). This process yielded six *ex-vivo* biopsies per patient: damaged mucosa (CDM), damaged submucosa (CDSM), damaged muscolaris/serosa (CDS), healthy mucosa (HM), healthy submucosa (HSM) and healthy muscolaris/serosa (HS).

The healthy tissue samples were taken >1 cm away from the visibly inflamed area. The classification of tissue as healthy was based on both macroscopic appearance and confirmation from the surgical pathology report, indicating no histopathologic abnormalities. Clinical samples were immediately preserved in NucleoProtect RNA (Macherey-Nagel, Germany) and stored at −80 °C for further analysis.

2.3. RNA extraction and Nanostring gene expression analysis

Total RNA was extracted from frozen ileal tissue using a NucleoSpin® RNA kit (Macherey-Nagel, Germany) according to the manufacturer's instructions. RNA concentration and purity were determined using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, USA), while RNA quality was assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, USA) and the RNA 6000 Nano LabChip Kit (Agilent Technologies, USA). In total, 50 ng of RNA was subjected to Nanostring gene expression analysis with the Nanostring nCounter system. The relative mRNA copies number of 579 human genes that are differentially expressed in immunological disorders was quantified as per the manufacturer's recommendations (NanoString Technologies, USA) using a nCounter® GX Human Immunology V2 kit (NanoString Technologies, USA).

2.3. Bioinformatic and statistical analysis

The raw gene expression data generated by the Nanostring system were processed using the nSolver™ Analysis Software 4.0 (NanoString Technologies, USA). To minimize the background noise, a background correction was performed using the geometric mean of negative controls. Data normalization was carried out in two steps: first, by normalizing to the geometric mean of six positive controls, and second, by normalizing to the geometric mean of 15 housekeeping genes (NanoString Technologies, USA). A comparative analysis of gene expression was then conducted by comparing counts from diseased ileal tissue sections to their adjacent healthy counterparts (*e.g.*, CDM *vs*. HM, CDSM *vs*. HSM, CDS *vs*. HS). Finally, a vertical analysis was conducted in both diseased and healthy ileum through pairwise comparisons (CDSM *vs*. CDM, CDS *vs*. CDM, and CDS *vs*. CDSM; HSM *vs*. HM, HS *vs* HM, and HS *vs*. HSM).

For statistical analysis, differential expression was determined using the nCounter Advanced Analysis 2.0.134 software (NanoString Technologies, USA), applying a Benjamini-Hochberg correction to adjust for multiple testing. For further differential expression analysis, only mRNAs with a $\log_2(fc) \geq 1$ or ≤ -1 and a BH-$p<$ 0,05 were deemed biologically and statistically significant, while mRNA with a $\log_2(fc) \geq 2$ or ≤ -2 and a BH-$p<$ 0,05 were considered strongly differentially expressed.

Graphical representations were generated using the nCounter Advanced Analysis 2.0.134 software (NanoString Technologies, USA) and GraphPad Prism version 8.4.3.686.

Data Availability Statement: The data underlying this study are not publicly available due to ethical and privacy considerations involving human subjects. However, they are available from the corresponding author upon reasonable request.

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CRediT authorship contribution statement

G Nannini: Conceptualization, Investigation, Visualization, Writing – original draft, Writing – review & editing, Formal analysis. **F Giudici:** Conceptualization, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **Dorian Fink:** Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **S Scaringi:** Investigation, Formal analysis. **F Cei:** Formal analysis, Investigation. **S Bertorello:** Formal analysis, Investigation. **Russo E:** Conceptualization, Investigation. **L Fortuna:** Formal analysis, Investigation. **F Staderini:** Formal analysis, Investigation. **F Cianchi:** Formal analysis, Investigation, Funding acquisition. **E Niccolai:** Data curation, Supervision, Validation, Writing – review & editing. **A Amedei:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing, Validation.

Declaration of competing interest

The authors declare they have no conflict of interests.

References

[1] J.H. Cho, The genetics and immunopathogenesis of inflammatory bowel disease, Nat. Rev. Immunol. 8 (6) (Jun. 2008) 458–466, https://doi.org/10.1038/nri2340.
[2] W. Strober, I. Fuss, P. Mannon, The fundamental basis of inflammatory bowel disease, J. Clin. Invest. 117 (3) (Mar. 2007) 514–521, https://doi.org/10.1172/JCI30587.
[3] J.T. Chang, Pathophysiology of inflammatory bowel diseases, N. Engl. J. Med. 383 (27) (Dec. 2020) 2652–2664, https://doi.org/10.1056/NEJMra2002697.
[4] L. Cervera-Seco, et al., Characterization of the regulatory landscape in Crohn's Disease reveals microRNA-associated alterations that shape anti-TNF response, Inflamm. Bowel Dis. (Mar. 2025), https://doi.org/10.1093/ibd/izaf029.
[5] N.M. Garcia, N.A. Cohen, D.T. Rubin, Treat-to-target and sequencing therapies in Crohn's disease, United Eur. Gastroenterol. J. 10 (10) (Dec. 2022) 1121–1128, https://doi.org/10.1002/ueg2.12336.
[6] Z. Liu, et al., Genetic architecture of the inflammatory bowel diseases across East Asian and European ancestries, Nat. Genet. 55 (5) (May 2023) 796–806, https://doi.org/10.1038/s41588-023-01384-0.
[7] H. Schmitt, M.F. Neurath, R. Atreya, Role of the IL23/IL17 pathway in Crohn's Disease, Front. Immunol. 12 (2021) 622934, https://doi.org/10.3389/fimmu.2021.622934.
[8] J. Torres, et al., ECCO guidelines on therapeutics in Crohn's disease: medical treatment, J. Crohns. Colitis 14 (1) (Jan. 2020) 4–22, https://doi.org/10.1093/ecco-jcc/jjz180.
[9] J.D. Feuerstein, et al., AGA clinical practice guidelines on the medical management of moderate to severe luminal and perianal fistulizing Crohn's Disease, Gastroenterology 160 (7) (Jun. 2021) 2496–2508, https://doi.org/10.1053/j.gastro.2021.04.022.
[10] T. Raine, et al., ECCO guidelines on therapeutics in ulcerative colitis: medical treatment, J. Crohns. Colitis 16 (1) (Jan. 2022) 2–17, https://doi.org/10.1093/ecco-jcc/jjab178.
[11] E. Russo, et al., Diving into inflammation: a pilot study exploring the dynamics of the immune-microbiota axis in ileal tissue layers of patients with Crohn's Disease, J. Crohn's Colitis 15 (9) (2021) 1500–1516, https://doi.org/10.1093/ecco-jcc/jjab034.
[12] R. Huang, et al., Identifying immune cell infiltration and effective diagnostic biomarkers in Crohn's disease by bioinformatics analysis, Front. Immunol. 14 (2023) 1162473, https://doi.org/10.3389/fimmu.2023.1162473.
[13] J.C. Coffey, D.P. O'Leary, The mesentery: structure, function, and role in disease, lancet. Gastroenterol. Hepatol. 1 (3) (Nov. 2016) 238–247, https://doi.org/10.1016/S2468-1253(16)30026-7.
[14] N.B. Kumar, T.T. Nostrant, H.D. Appelman, The histopathologic spectrum of acute self-limited colitis (acute infectious-type colitis), Am. J. Surg. Pathol. 6 (6) (Sep. 1982) 523–529, https://doi.org/10.1097/00000478-198209000-00004.
[15] B.M. Fournier, C.A. Parkos, The role of neutrophils during intestinal inflammation, Mucosal Immunol 5 (4) (Jul. 2012) 354–366, https://doi.org/10.1038/mi.2012.24.
[16] H. Chen, X. Wu, C. Xu, J. Lin, Z. Liu, Dichotomous roles of neutrophils in modulating pathogenic and repair processes of inflammatory bowel diseases, Precis. Clin. Med. 4 (4) (Dec. 2021) 246–257, https://doi.org/10.1093/pcmedi/pbab025.
[17] C. Abraham, J.H. Cho, Inflammatory bowel disease, N. Engl. J. Med. 361 (21) (Nov. 2009) 2066–2078, https://doi.org/10.1056/NEJMra0804647.

[18] M.S. Alexdottir, et al., Neutrophil-mediated type IV collagen degradation is elevated in patients with mild endoscopic ulcerative colitis reflecting early mucosal destruction, Sci. Rep. 14 (1) (2024) 1641, https://doi.org/10.1038/s41598-024-52208-y.
[19] M.A. Alzoghaibi, Neutrophil expression and infiltration into Crohn's intestine, Saudi J. Gastroenterol. Off. J. Saudi Gastroenterol. Assoc. 11 (2) (May 2005) 63–72, https://doi.org/10.4103/1319-3767.33322.
[20] O. Wéra, P. Lancellotti, C. Oury, The Dual Role of Neutrophils in Inflammatory Bowel Diseases, J. Clin. Med. 5 (12) (2016), https://doi.org/10.3390/jcm5120118.
[21] S. Bernhard, et al., Interleukin 8 elicits rapid physiological changes in neutrophils that are altered by inflammatory conditions, J. Innate Immun. 13 (4) (2021) 225–241, https://doi.org/10.1159/000514885.
[22] T. Kucharzik, et al., Acute induction of human IL-8 production by intestinal epithelium triggers neutrophil infiltration without mucosal injury, Gut 54 (11) (Nov. 2005) 1565–1572, https://doi.org/10.1136/gut.2004.061168.
[23] C. Bizzarri, A.R. Beccari, R. Bertini, M.R. Cavicchia, S. Giorgini, M. Allegretti, ELR + CXC chemokines and their receptors (CXC chemokine receptor 1 and CXC chemokine receptor 2) as new therapeutic targets, Pharmacol. Ther. 112 (1) (Oct. 2006) 139–149, https://doi.org/10.1016/j.pharmthera.2006.04.002.
[24] A.A. Kühl, et al., Aggravation of different types of experimental colitis by depletion or adhesion blockade of neutrophils, Gastroenterology 133 (6) (Dec. 2007) 1882–1892, https://doi.org/10.1053/j.gastro.2007.08.073.
[25] C. Danne, J. Skerniskyte, B. Marteyn, H. Sokol, Neutrophils: from IBD to the gut microbiota, Nat. Rev. Gastroenterol. Hepatol. 21 (3) (2024) 184–197, https://doi.org/10.1038/s41575-023-00871-3.
[26] S.G. Meuwissen, T.M. Feltkamp-Vroom, A.B. De La Rivière, A.E. Von Dem Borne, G. N. Tytgat, Analysis of the lympho-plasmacytic infiltrate in Crohn's disease with special reference to identification of lymphocyte-subpopulations, Gut 17 (10) (Oct. 1976) 770–780, https://doi.org/10.1136/gut.17.10.770.
[27] C. Smids, et al., Intestinal T cell profiling in inflammatory bowel disease: linking t cell subsets to disease activity and disease course, J. Crohns. Colitis 12 (4) (Mar. 2018) 465–475, https://doi.org/10.1093/ecco-jcc/jjx160.
[28] E.J. Wherry, T cell exhaustion, Nat. Immunol. 12 (6) (2011) 492–499, https://doi.org/10.1038/ni.2035.
[29] E.F. McKinney, J.C. Lee, D.R.W. Jayne, P.A. Lyons, K.G.C. Smith, T-cell exhaustion, co-stimulation and clinical outcome in autoimmunity and infection, Nature 523 (7562) (2015) 612–616, https://doi.org/10.1038/nature14468.
[30] R.C. Garduño and J. Däbritz, "New insights on CD8 + T Cells in In fl ammatory bowel disease and therapeutic approaches," vol. 12, no. October, pp. 1–15, 2021, doi: 10.3389/fimmu.2021.738762.
[31] A. Saez, B. Herrero-Fernandez, R. Gomez-Bris, H. Sánchez-Martinez, J. M. Gonzalez-Granado, Pathophysiology of inflammatory bowel disease: innate immune system, Int. J. Mol. Sci. 24 (2) (Jan. 2023), https://doi.org/10.3390/ijms24021526.
[32] T. Castro-Dopico, M.R. Clatworthy, Mucosal IgG in inflammatory bowel disease - a question of (sub)class? Gut Microbes 12 (1) (Nov. 2020) 1–9, https://doi.org/10.1080/19490976.2019.1651596.
[33] A.R. Bourgonje, et al., Patients with inflammatory bowel disease show IgG immune responses towards specific intestinal bacterial genera, Front. Immunol. 13 (2022) 842911, https://doi.org/10.3389/fimmu.2022.842911.
[34] R. Zogorean, S. Wirtz, The yin and yang of B cells in a constant state of battle: intestinal inflammation and inflammatory bowel disease, Front. Immunol. 14 (2023) 1260266, https://doi.org/10.3389/fimmu.2023.1260266.
[35] R. Dheer, et al., Microbial signatures and innate immune gene expression in lamina propria phagocytes of inflammatory bowel disease patients, Cell. Mol. Gastroenterol. Hepatol. 9 (3) (2020) 387–402, https://doi.org/10.1016/j.jcmgh.2019.10.013.
[36] J.-H. Han, et al., Effective anti-tumor response by TIGIT blockade associated with FcγR engagement and myeloid cell activation, Front. Immunol. 11 (2020) 573405, https://doi.org/10.3389/fimmu.2020.573405.
[37] F. Jones, G. Doherty, E. McNamee, P071 Crohn's Disease is associated with elevated levels of the pro-inflammatory CXCR3 ligands (CXCL9, 10 and 11) with an associated reduction in Paneth cell derived antimicrobial peptides in ex-vivo ileal biopsies, J. Crohn's Colitis 15 (Supplement_1) (2021) S174–S175, https://doi.org/10.1093/ecco-jcc/jjab076.200.
[38] M. Walshe, et al., A Role for CXCR3 ligands as biomarkers of post-operative Crohn's Disease recurrence, J. Crohns. Colitis 16 (6) (Jul. 2022) 900–910, https://doi.org/10.1093/ecco-jcc/jjab186.
[39] U.P. Singh, C. Venkataraman, R. Singh, J.W.J. Lillard, CXCR3 axis: role in inflammatory bowel disease and its therapeutic implication, Endocr. Metab. Immune Disord. Drug Targets 7 (2) (Jun. 2007) 111–123, https://doi.org/10.2174/187153007780832109.
[40] B. Chami, A.W.S. Yeung, C. van Vreden, N.J.C. King, S. Bao, The Role of CXCR3 in DSS-induced colitis, PLoS One 9 (7) (2014) 1–9, https://doi.org/10.1371/journal.pone.0101622.
[41] H.M. Isles, et al., The CXCL12/CXCR4 signaling axis retains neutrophils at inflammatory sites in Zebrafish, Front. Immunol. 10 (2019) 1784, https://doi.org/10.3389/fimmu.2019.01784.
[42] I. Dotan, et al., CXCL12 Is a constitutive and inflammatory chemokine in the intestinal immune system, Inflamm. Bowel Dis. 16 (4) (2009) 583–592, https://doi.org/10.1002/ibd.21106.
[43] A.S. Vamadevan, M. Fukata, E.T. Arnold, L.S. Thomas, D. Hsu, M.T. Abreu, Regulation of Toll-like receptor 4-associated MD-2 in intestinal epithelial cells: a comprehensive analysis, Innate Immun 16 (2) (Apr. 2010) 93–103, https://doi.org/10.1177/1753425909339231.
[44] M.T. Abreu, et al., TLR4 and MD-2 expression is regulated by immune-mediated signals in human intestinal epithelial cells, J. Biol. Chem. 277 (23) (Jun. 2002) 20431–20437, https://doi.org/10.1074/jbc.M110333200.
[45] P.L. Beatty, S.E. Plevy, A.R. Sepulveda, O.J. Finn, Cutting edge: transgenic expression of human MUC1 in IL-10-/- mice accelerates inflammatory bowel disease and progression to colon Cancer1, J. Immunol. 179 (2) (2007) 735–739, https://doi.org/10.4049/jimmunol.179.2.735.
[46] J.G. Hashash, et al., Altered expression of the epithelial mucin MUC1 accompanies endoscopic recurrence of postoperative Crohn's Disease, J. Clin. Gastroenterol. 55 (2) (Feb. 2021) 127–133, https://doi.org/10.1097/MCG.0000000000001340.
[47] Y. Kida, et al., Urokinase-type plasminogen activator blockade ameliorates experimental colitis in mice, Sci. Rep. 13 (1) (Feb. 2023) 2899, https://doi.org/10.1038/s41598-023-29824-1.
[48] E. Proietti, et al., Modulation of indoleamine 2,3-dioxygenase 1 during inflammatory bowel disease activity in humans and mice, Int. J. Tryptophan Res. 16 (2023) 11786469231153108, https://doi.org/10.1177/11786469231153109.
[49] T.M. Connelly, et al., T-cell activation Rho GTPase-activating protein expression varies with inflammation location and severity in Crohn's disease, J. Surg. Res. 190 (2) (2014) 457–464, https://doi.org/10.1016/j.jss.2014.01.019.
[50] D.E. Levy et al., "Socs3 expression in myeloid cells modulates the pathogenesis of dextran sulfate sodium (DSS) - induced colitis," no. May, pp. 1–12, 2023, doi: 10.3389/fimmu.2023.1163987.
[51] Y. Li, et al., Disease-related expression of the IL6/STAT3/SOCS3 signalling pathway in ulcerative colitis and ulcerative colitis-related carcinogenesis, Gut 59 (2) (Feb. 2010) 227–235, https://doi.org/10.1136/gut.2009.184176.
[52] Y. Miyanaka, et al., Clinical significance of mucosal suppressors of cytokine signaling 3 expression in ulcerative colitis, World J. Gastroenterol. 13 (21) (Jun. 2007) 2939–2944, https://doi.org/10.3748/wjg.v13.i21.2939.
[53] M.A.H. Ahmed, et al., CD24 is upregulated in inflammatory bowel disease and stimulates cell motility and colony formation, Inflamm. Bowel Dis. 16 (5) (May 2010) 795–803, https://doi.org/10.1002/ibd.21134.
[54] P. Fernandes, et al., Identifying metabolic shifts in Crohn's disease using 'omics-driven contextualized computational metabolic network models, Sci. Rep. 13 (1) (2023) 203, https://doi.org/10.1038/s41598-022-26816-5.
[55] N. Imazu, et al., Arginase 2 ameliorates experimental colitis through antioxidant effects via spermidine production, Inflamm. Bowel Dis. 30 (Supplement_1) (2024) S2–S3, https://doi.org/10.1093/ibd/izae020.005.
[56] N. Imazu, et al., Arginase 2 attenuates ulcerative colitis by antioxidant effects of spermidine, J. Gastroenterol. 59 (8) (Aug. 2024) 682–698, https://doi.org/10.1007/s00535-024-02104-z.
[57] J.-Y. Li, et al., Arginine metabolism regulates the pathogenesis of inflammatory bowel disease, Nutr. Rev. 81 (5) (Apr. 2023) 578–586, https://doi.org/10.1093/nutrit/nuac070.
[58] L. Zhao et al., "CD36 Senses Dietary Lipids and Regulates Lipids Homeostasis in the Intestine," vol. 12, no. April 2021, doi: 10.3389/fphys.2021.669279.
[59] V. Cifarelli, et al., CD36 deficiency impairs the small intestinal barrier and induces subclinical inflammation in mice, Cell. Mol. Gastroenterol. Hepatol. 3 (1) (Jan. 2017) 82–98, https://doi.org/10.1016/j.jcmgh.2016.09.001.
[60] I. Angriman, et al., Innate immunity activation in newly diagnosed ileocolonic Crohn's Disease: a cohort study, Dis. Colon Rectum 67 (5) (May 2024) 681–692, https://doi.org/10.1097/DCR.0000000000003145.