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The Role of IgE in Crohn's Disease by Impairing the Capacity of Plasmacytoid Dendritic Cells to Generate FOXP3$^+$ Tregs

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Keywords: Crohn's disease | IgE | plasmacytoid dendritic cells | regulatory T cells

ABSTRACT

Background: A causal relationship between Crohn's disease (CD) and asthma is reported, but the underlying mechanisms are not fully understood. We sought to investigate the role of IgE and IgE-mediated pathways in the pathophysiology of CD.

Methods: 20 CD patients, 10 allergic patients without inflammatory bowel disease, and 10 healthy donors (HD) were included in the study. Total serum IgE was quantified by ELISA. Circulating IgE$^+$ and FcεRIα$^+$ immune cells, as well as specific CD4$^+$ T cell populations, were determined by flow cytometry. Gene set enrichment signatures from available single-cell (sc)RNAseq datasets of the intestine from CD patients were analyzed. Purified plasmacytoid dendritic cells (pDCs) from CD patients were cocultured with naïve CD4$^+$ T cells to assess Tregs generation.

Results: CD patients, similar to allergic non-CD patients, displayed significantly higher numbers of circulating IgE$^+$ or FcεRIα$^+$ immune cells than HD. The percentage of blood IgE$^+$ or FcεRIα$^+$ pDCs was significantly higher in CD than HD and similar to allergic non-CD patients. CD patients showed significantly higher numbers of effector memory CD4$^+$ T cells and lower numbers of FOXP3$^+$ Tregs than HD. scRNAseq data from CD patients confirmed that Tregs imbalance and overactivation of IgE-mediated pathways take place also in gut tissues of children and adults, suggesting IgE could interfere in the pDC-Tregs axis. In vitro

Abbreviations: CD, Crohn's disease; CL, crosslinker; CRSwNP, chronic rhinosinusitis with nasal polyps; CSU, chronic spontaneous urticaria; FcεR, IgE receptor; FlowSOM, flow self-organizing map; HBI, Harvey-Bradshaw index; HD, healthy donors; IBD, inflammatory bowel disease; IC, isotype control; IgE, immunoglobulin E; IL, interleukin; mAb, monoclonal antibody; mDC, myeloid dendritic cells; MFI, mean fluorescence intensity; PBMCs, peripheral blood mononuclear cells; pDCs, plasmacytoid dendritic cells; sc, single-cell; TLR9-L, TLR9-ligand; TMB, tetramethylbenzidine; TNF, tumor necrosis factor; Tregs, regulatory T cells; UMAP, uniform manifold approximation and projection.

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functional experiments demonstrated that IgE-crosslinking on pDCs from CD patients impairs Treg generation, which was restored by the anti-IgE mAb omalizumab.

Conclusions: IgE might play an unprecedented role in CD by impairing the capacity of pDCs to generate Tregs, which could represent a novel mechanism contributing to CD to be exploited for alternative therapeutic interventions.

1 | Introduction

Inflammatory bowel disease (IBD) is a family of autoimmune, chronic, idiopathic, and relapsing disorders of increasing prevalence worldwide that represent a global public health challenge [1–3]. IBD encompasses two different disorders: Crohn's disease (CD) and ulcerative colitis. CD is characterized by recurrent destructive pathological inflammation causing progressive gut damage [3]. Clinical presentation depends on disease location and behavior, as well as the severity of the inflammation [4]. Over the last decades, the therapeutic landscape for CD has broadened [5]. Monoclonal antibodies (mAbs) targeting single pro-inflammatory cytokines or integrins, such as tumor necrosis factor (TNF) inhibitors, integrin inhibitors, and interleukin (IL)-12/23 inhibitors, are considered the mainstay of treatment for CD. However, a high rate of patients receiving biologics do not respond to induction or eventually have a loss of response, often due to anti-drug antibody formation. This remains a significant therapeutic challenge and opens up the opportunity for new advanced treatments involving other mechanisms of action [6].

An increasing number of studies have focused on the potential association between CD and asthma, and recent substantial evidence supports a causal relationship between both [7–9]. A crosstalk between the gastrointestinal and respiratory tracts (termed the gut-lung axis) has been described, but the potential molecular mechanisms underlying the CD and asthma association remain elusive [9–11]. The identification of common immune pathways contributing to this simultaneous occurrence is of utmost importance not only to confirm the link between CD and asthma but also to develop novel specific interventions. In this regard, although the pivotal role of immunoglobulin E (IgE) in asthma is widely acknowledged and there is growing evidence suggesting that IgE plays a role also in autoimmune diseases, its actual implication in CD is not yet fully understood [12, 13]. Most of the studies investigating the potential link between IgE and CD have mainly focused on evaluating whether there is a higher incidence of allergic diseases in CD patients than in healthy individuals [14–17]. Indeed, an increased IgE sensitization against food allergens and aeroallergens has been generally reported for CD patients, particularly in those with orofacial granulomatosis, but the actual contribution of IgE in CD is still controversial [18, 19].

IgE is synthesized and secreted by B cells and exerts its functional properties by binding to either the high-affinity IgE receptor (FcεRI) or to CD23 (also known as FcεRII), which are expressed on a plethora of immune cells, including basophils, mast cells, B cells, or plasmacytoid dendritic cells (pDCs), among others [20]. Along with increased IgE levels, reduced percentages of regulatory T cells (Tregs) are a hallmark both in CD and asthma [21–24]. Loss of functional Tregs disrupts homeostasis and is linked to exacerbated inflammatory responses in asthma and other autoimmune diseases such as CD [25, 26]. Of note, in the context of asthma, we demonstrated that IgE-FcεRI cross-linking on pDCs impairs the generation of Tregs, which could be restored in vitro by omalizumab, an anti-IgE mAb approved for the treatment of severe allergic asthma [27, 28]. Supporting these data, the administration of omalizumab to children clinically improves asthma control by restoring Tregs homeostasis [29]. Whether similar mechanisms might take place also in the context of CD remains fully elusive.

Herein, we sought to investigate the potential role of IgE and IgE-mediated pathways in the pathophysiology of CD. Our data provide novel molecular insights into the contribution of IgE^+ pDCs to inflammation in CD by compromising Tregs homeostasis, which might well open new avenues to be exploited for alternative therapeutic interventions.

2 | Materials and Methods

2.1 | Study Design and Patient Population

This was a unicentre, cross-sectional cohort study of CD patients. The study population included patients aged ≥18 years with an established diagnosis of CD according to Lennard-Jones criteria, who are in clinical remission (Harvey-Bradshaw index [HBI] ≤4). At the time of the study, patients may or may not receive maintenance therapy with immunomodulators or mesalamine according to standard clinical practice. Patients receiving biologics or with active CD (HBI >4) were excluded. CD patients were classified by phenotype according to Montreal classification [30]. CD patients were age and sex-matched with a control HD group. A cohort of allergic patients without CD (allergic non-CD patients) was also recruited and included in the study for comparison purposes.

The study was performed according to the Declaration of Helsinki's ethical guidelines and was approved by the Clinical Research Ethics Committees of the participating centers (leading center: Hospital Universitario Clínico San Carlos, Madrid, Spain; C.I. 21/567-E, 8 September 2021). All enrolled patients and controls provided written consent for their participation in the study.

All procedures used in this study are fully described in the Methods section in this article's Supporting Information and Supporting Information Tables 1 and 2.

3 | Results

3.1 | Patients and Control Populations

To investigate the potential contribution of IgE and IgE-mediated signaling pathways in Crohn's disease (CD), 20 patients diagnosed with CD, 10 allergic non-CD patients,

and 10 healthy donors (HD) were recruited from December 2021 to October 2024 at the Gastroenterology Department of Hospital Clínico San Carlos, Madrid, Spain (CD patients) or at the Allergy Department of Fundación Jiménez Díaz, Madrid, Spain (allergic non-CD patients). Their demographic and clinical features are collected in Table 1. The mean age of the CD patients was 44 years, and around 65% of them were male. Most of the patients had ileal or ileal-colon CD, 70% had a non-stricturing, non-penetrating disease behavior, and 40% were receiving immunosuppressants. All patients were in remission at the time of the sample collection. Ten patients (50%) had associated IgE-mediated comorbidities, including allergic asthma, rhinitis, or rhinoconjunctivitis. Allergic non-CD patients were diagnosed based on their clinical history, positive skin prick test reactions, and specific IgE level measurement. All of the patients had rhinoconjunctivitis or rhinitis, concomitant in some cases with allergic asthma, food allergy, or drug allergy (Table 1).

3.2 | Patients Suffering from Crohn's Disease, Similar to Allergic non-CD Individuals, Display Significantly Higher Numbers of Circulating IgE+ or FcεRIα+ Immune Cells than Healthy Donors

We first quantified and compared the levels of serum total IgE in CD patients, allergic non-CD patients, and HD. A trend towards higher levels of circulating total free IgE was observed in CD and allergic non-CD patients compared to HD (Figure 1A). IgE performs its functional properties upon binding to the high-affinity IgE-receptor subunit α (FcεRIα) on a plethora of immune system cells. We isolated peripheral blood mononuclear cells (PBMCs) from CD patients, allergic non-CD individuals, and HD to quantify and compare by flow cytometry the frequencies of IgE^+ or $FcεRIα^+$ immune cells. As shown in Figure 1B, the percentages of circulating IgE^+ or $FcεRIα^+$ immune cells were significantly higher in PBMCs from CD patients and allergic non-CD patients than in HD, without significant differences between CD and allergic non-CD patients. In addition, CD patients and allergic non-CD patients also displayed a significantly higher number of surface-bound IgE molecules within the total IgE^+ cell fraction than HD, as determined by mean fluorescence intensity (MFI) quantification (Figure 1C). A moderate but significant positive correlation was observed between serum total IgE levels and IgE^+ cells within PBMCs when using the samples from all the cohorts in a single correlation analysis, supporting our previous data showing that the percentages of circulating IgE^+ cells are related to the total serum IgE levels (Figure 1D). To rule out a possible bias due to the fact that 50% of the CD patients included in this study had concomitant allergic diseases, we stratified patients according to their allergic status and compared total serum IgE levels as well as IgE^+ or $FcεRIα^+$ cells in PBMCs. Allergic and non-allergic CD patients showed a tendency towards higher serum total IgE levels than HD (Supporting Information Figure 1A). Notably, CD patients, regardless of their allergic status, showed significantly higher frequencies of IgE^+ or $FcεRIα^+$ cells than HD (Supporting Information Figure 1B). Supporting these data, CD patients displayed a significantly higher number of surface-bound IgE molecules within the total IgE^+ cell fraction than HD, with no significant differences observed when stratifying CD patients according to their allergic status (Supporting Information Figure 1C).

Collectively, these data demonstrate that CD patients, as well as allergic non-CD individuals, display significantly higher frequencies of circulating IgE^+ or $FcεRIα^+$ immune cells than HD. Importantly, the observed increased percentages do not depend on the concomitance of allergic diseases in CD patients.

3.3 | The Frequency of Circulating Basophils, pDCs, and mDCs 2 Loaded With IgE on Their Surfaces Is Enriched in Crohn's Disease Patients Compared to Healthy Donors

To gain further insights into the potential contribution of different IgE^+ immune cells to the pathophysiology of CD, we identified specific cell subsets with canonical markers by FlowSOM clusters within the 2D UMAP plot (Figure 2A). Six different immune cell populations were identified, including pDCs, type 1 myeloid DCs (mDCs 1), basophils, B cells, type 2 myeloid DCs (mDCs 2), and monocytes (Figure 2A). For a detailed analysis of IgE sensitization and surface FcεRIα expression in basophils, pDCs, and mDCs 2 as key cells involved in IgE-mediated inflammation and antigen presentation, [13]. flow cytometry data from PBMCs of the 20 CD patients, 10 allergic non-CD patients, and the 10 HD were manually gated following the strategy shown in Supporting Information Figure 2. The percentage of circulating basophils bearing IgE or FcεRIα on their surface was significantly higher in CD patients than HD and similar to that observed in allergic non-CD patients (Figures 2B,C). pDCs represent a DC subset with intrinsic tolerogenic capacity that is able to polarize functional Tregs. IgE-FcεRI cross-linking on pDCs from atopic donors impairs the capacity of pDCs to generate Tregs, which can be restored by anti-IgE treatments [27]. Remarkably, CD patients displayed significantly higher percentages of circulating IgE^+ or $FcεRIα^+$ pDCs than HD, reaching levels equal to those displayed by allergic non-CD individuals (Figures 2B,C). Furthermore, we also analyzed and compared mDCs 2, a DC subset that expresses FcεRIα able to bind IgE and play an important role in T cell activation and polarization. The frequency of mDCs 2 bearing IgE or FcεRIα on their surface was also significantly increased in CD patients compared to HD, with similar frequencies observed when comparing with allergic non-CD patients (Figure 2B,C). Collectively, these data indicate that the frequencies of circulating IgE^+ or $FcεRIα^+$ basophils, pDCs, and mDCs 2 are significantly increased in CD patients compared to HD, suggesting that they could also contribute to the pathophysiology of CD.

3.4 | Crohn's Disease Patients Showed Lower Circulating $FOXP3^+$ Tregs and Higher Effector Memory $CD4^+$ T Cells Than Healthy Donors

To investigate whether the significantly higher levels of IgE^+ pDCs observed in CD patients could be concomitant with

TABLE 1 | Demographics and clinical characteristics of participants.

	CD patients	Allergic patients[#]	Healthy donors[+]
	***n* = 20**	***n* = 10**	***n* = 10**
Age, years, mean (SD)	44.1 (9.8)	34 (6.5)	43 (8.6)
Sex, male, *n* (%)	13 (65)	6 (60)	7 (70)
IBD family history, *n* (%)	4 (20)	0 (0)	0 (0)
Age at CD diagnosis*		N/A	N/A
≤ 16 years [A1], *n* (%)	2 (10)		
17–40 years [A2], *n* (%)	17 (85)		
≥ 40 years [A3], *n* (%)	1 (5)		
Location*		N/A	N/A
Ileal [L1], *n* (%)	10 (50)		
Colonic [L2], *n* (%)	3 (15)		
Ileocolonic [L3], *n* (%)	7 (35)		
Behavior*		N/A	N/A
Non-stricturing, non-penetrating [B1], *n* (%)	14 (70)		
Stricturing [B2], *n* (%)	2 (10)		
Penetrating [B3], *n* (%)	4 (20)		
Perianal disease [p], *n* (%)	3 (15)		
Smoker status		N/A	N/A
Current, *n* (%)	2 (10)		
Past, *n* (%)	10 (50)		
Never, *n* (%)	8 (40)		
Crohn's disease surgical history		N/A	N/A
Prior surgical treatment, *n* (%)	5 (25)		
Prior treatment		N/A	N/A
Immunosuppressant, *n* (%)	1 (5)		
Biological therapy (adalimumab), *n* (%)	2 (10)		
Mesalamine, *n* (%)	10 (50)		
Current treatment		N/A	N/A
Immunosuppressant, *n* (%)	8 (40)		
Mercaptopurine, *n* (%)	5 (62.5)		
Azathioprine, *n* (%)	3 (37.5)		
Mesalamine, *n* (%)	4 (20)		
No maintenance therapy, *n* (%)	8 (40)		
IgE-mediated diseases, *n* (%)	10 (50)	10 (100)	0 (0)
Rhinoconjunctivitis, *n* (%)	7 (35)	8 (80)	0 (0)
Bronchial asthma, *n* (%)	2 (10)	1 (10)	0 (0)
Rhinitis, *n* (%)	2 (10)	2 (20)	0 (0)
Food allergy, *n* (%)	0 (0)	1 (10)	0 (0)
Drug allergy, *n* (%)	0 (0)	1 (10)	0 (0)

Abbreviation: not applicable.
*Crohn's disease phenotype evaluated using the Montreal classification.
[#]None of the allergic patients had inflammatory bowel disease.
[+]None of the healthy donors had inflammatory bowel disease or allergy.

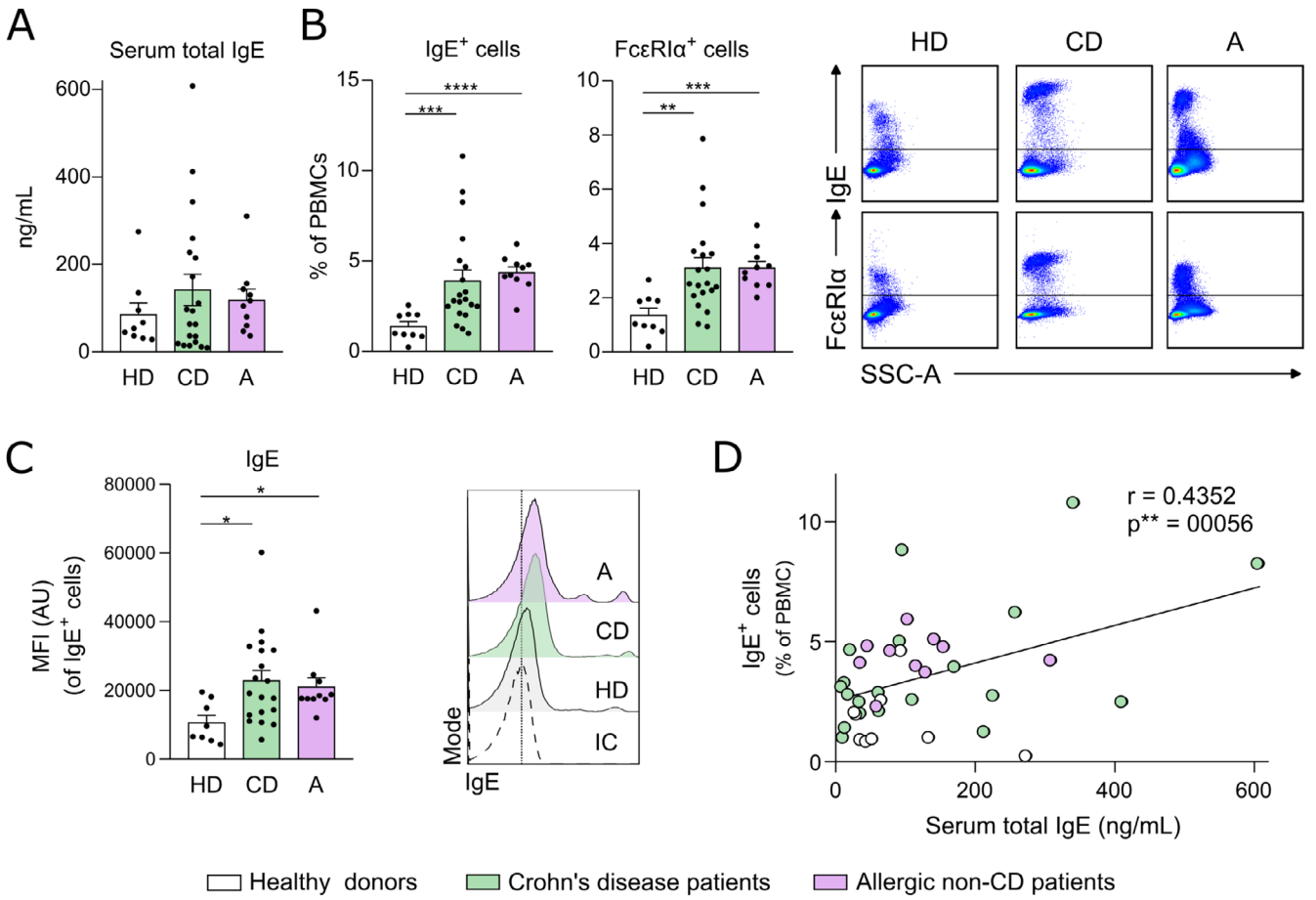

FIGURE 1 | CD patients display higher numbers of circulating IgE$^+$ and FcεRIα$^+$ immune cells than HD. (A) Serum total IgE levels in HD, CD and A patients as determined by ELISA. (B) Percentage of total IgE$^+$ and FcεRIα$^+$ immune cells in PBMCs from HD, CD and A patients as determined by flow cytometry. Right side, representative dot plots of IgE$^+$ and FcεRIα$^+$ cells from each group. (C) MFI (AU, arbitrary units) of IgE on IgE$^+$ immune cells in PBMCs from HD, CD and A patients. Right side, representative histograms of IgE expression in IgE$^+$ cells in PBMCs from each group compared to the isotype control. (D) Serum total IgE levels vs. the frequency of IgE$^+$ cells in PBMCs from all participants. "r" Pearson correlation coefficient. n (HD) = 8–9, n (CD) = 19–20, n (A) = 10. HD: Healthy donors, CD: Crohn's disease patients, A: Allergic non-CD patients. Mann–Whitney test or unpaired Student's *t* test; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ and ****$p < 0.0001$.

alterations at the T cell level, we identified and compared different T cell subsets according to the expression of relevant markers (Tregs, naïve and effector memory CD4$^+$ T cells) by the FlowSOM algorithm projected onto a UMAP plot (Figure 3A). We also analyzed these T cell subsets by manual gating, as shown in Supporting Information Figures 3 and 4. Remarkably, the percentage of circulating CD4$^+$CD127$^-$CD25$^+$FOXP3$^+$ Tregs was significantly lower in CD patients than in HD, with no significant differences observed when comparing with allergic non-CD patients (Figure 3B). In addition, CD patients displayed a significantly lower frequency of circulating naïve CD4$^+$ T cells (Figure 3C) and a significantly higher frequency of effector memory CD4$^+$ T cells (Figure 3D) than HD or allergic non-CD patients.

Collectively, these data demonstrate an imbalance between regulatory and effector functions at the T cell level in CD patients, a profile that is different from that observed both in HD and allergic non-CD patients.

3.5 | scRNAseq Analysis Reveals Exacerbated IgE-FcεRI Signaling in pDCs and Imbalanced Th Responses in the Gut of Crohn's Disease Patients

To assess whether our results could also have an impact on gut tissue from CD patients, we analyzed gene set enrichment signatures from publicly available scRNAseq datasets of intestinal samples from adult [31]. and pediatric CD patients [32]. (Figure 4). Comparative analysis of gene signature profiles within the annotated immune cells infiltrating gut tissues from adult CD patients (Figure 4A) showed that IgE-related genes were upregulated, indicating abnormal circulating IgE, higher activation of FcεRI signaling pathways, and increased DC activation in CD patients compared to HD (Figure 4B). Analysis of specific gene signatures from gut-resident T cells revealed increased Th1 and Th17 responses concomitant with a higher Th2 profile and a slight deficiency of Tregs in adult CD patients compared to HD (Figure 4C). Moreover, FcεRI contributions were analyzed in all DC-related cells, showing higher expression of the FcεRI signaling pathway

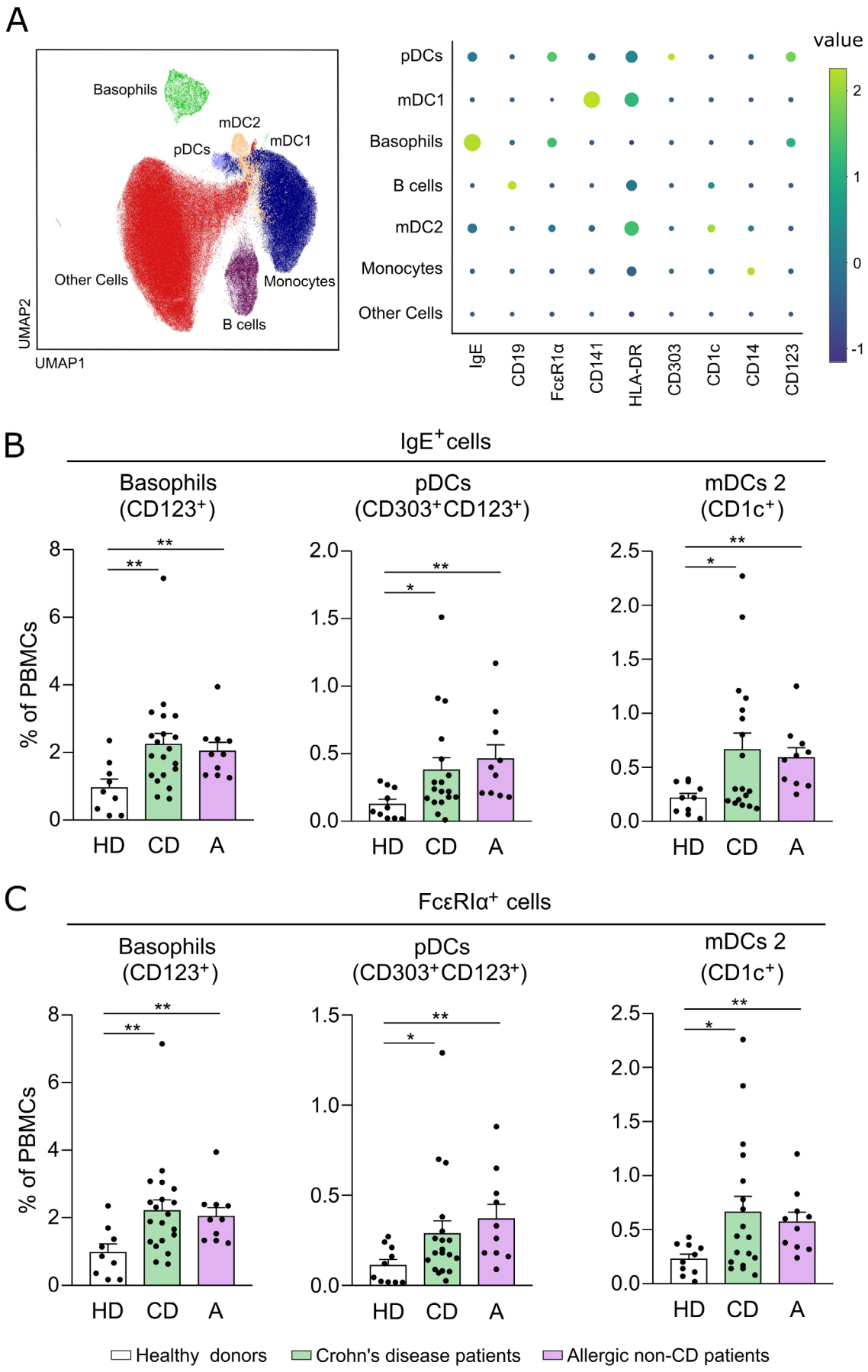

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FIGURE 2 | Basophils and DCs from CD patients are sensitized with IgE to a greater extent than those from HD. (A) Identification of immune cell populations from participants using Flow-SOM algorithm. Left side, representative UMAP plot showing identified populations. Right side, bubble heat map showing the expression of the markers used for the identification of each immune cell population. The color and size of the circles indicate the intensity and relative number of cells expressing each marker, respectively. (B) Percentage of IgE$^+$ basophils, pDCs, and mDCs 2 from HD, CD and A patients. (C) Percentage of FcεRIα$^+$ basophils, pDCs, and mDCs 2 from HD, CD and A patients. n (HD) = 9–10, n (CD) = 18–20, n (A) = 10. HD: Healthy donors, CD: Crohn's disease patients, A: Allergic non-CD patients. Mann–Whitney test or unpaired Student's *t* test; *$p < 0.05$ and **$p < 0.01$.

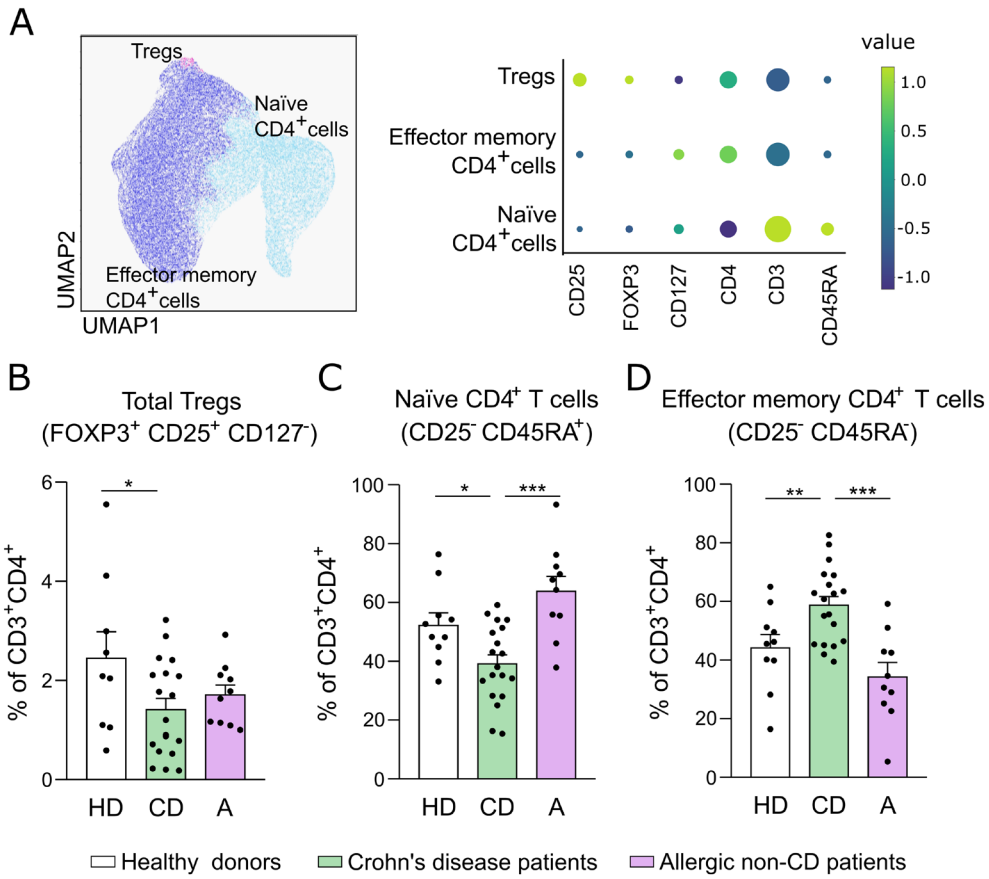

FIGURE 3 | CD patients display lower circulating Tregs and higher effector memory T cells than HD. (A) Identification of CD3$^+$CD4$^+$ cell populations from participants using Flow-SOM algorithm. Left side, representative UMAP plot showing identified populations. Right side, bubble heat map showing the expression of the markers used for the identification of each CD3$^+$CD4$^+$ T cell population. The color and size of the circles indicate the intensity and relative number of CD3$^+$CD4$^+$ T cells expressing each marker, respectively. Frequency of Tregs (B), naïve CD4$^+$ T cells (C), or effector memory CD4$^+$ T cells (D) in peripheral blood from HD, CD and A patients. n (HD) = 9–10, n (CD) = 19 n (A) = 10. HD: Healthy donors, CD: Crohn's disease patients, A: Allergic non-CD patients. Unpaired Student's *t* test; *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$.

and IgE-related genes (Figure 4D,E). The detailed analysis of gene signatures from all the annotated gut cells from pediatric CD patients (Figure 4F) uncovered that T cell responses in intestinal samples were equivalent to those observed in adults (Figure 4G). Interestingly, the mean expression of genes associated with the regulation of isotype switching to IgE in plasma cells was higher in CD patients than in HD (Figure 4H). Accordingly, a higher expression of IgE-related genes in B cells, such as those involved in IgE binding or IgE complex formation, was also observed in CD patients compared to HD (Figure 4I). Of note, intestinal pDCs from pediatric CD patients expressed higher levels of the high-affinity IgE-receptor subunit γ, *FCER1G* (Figure 4J), and increased gene signatures for pathways related to IgE binding and TNF production compared to HD (Figure 4K). These data suggest that pDCs in the gut of CD patients bind high levels of IgE through FcεRI and contribute to TNF-mediated inflammatory responses. Collectively, these results confirm that pDCs infiltrating gut tissues of CD patients might also show a significant activation of IgE-mediated signaling pathways potentially contributing to inflammatory local T cell responses by impairing the generation of Tregs.

3.6 | IgE-FcεRI Cross-Linking on pDCs From Crohn's Disease Patients Impair Their Capacity to Generate FOXP3$^+$ Tregs, Which Is Restored by the Anti-IgE mAb Omalizumab

To investigate the capacity of pDCs from CD patients to generate Tregs in vitro upon IgE-FcεRI cross-linking and the ability of omalizumab to influence such capacity, we purified pDCs from PBMCs of the CD patients included in this study. Freshly

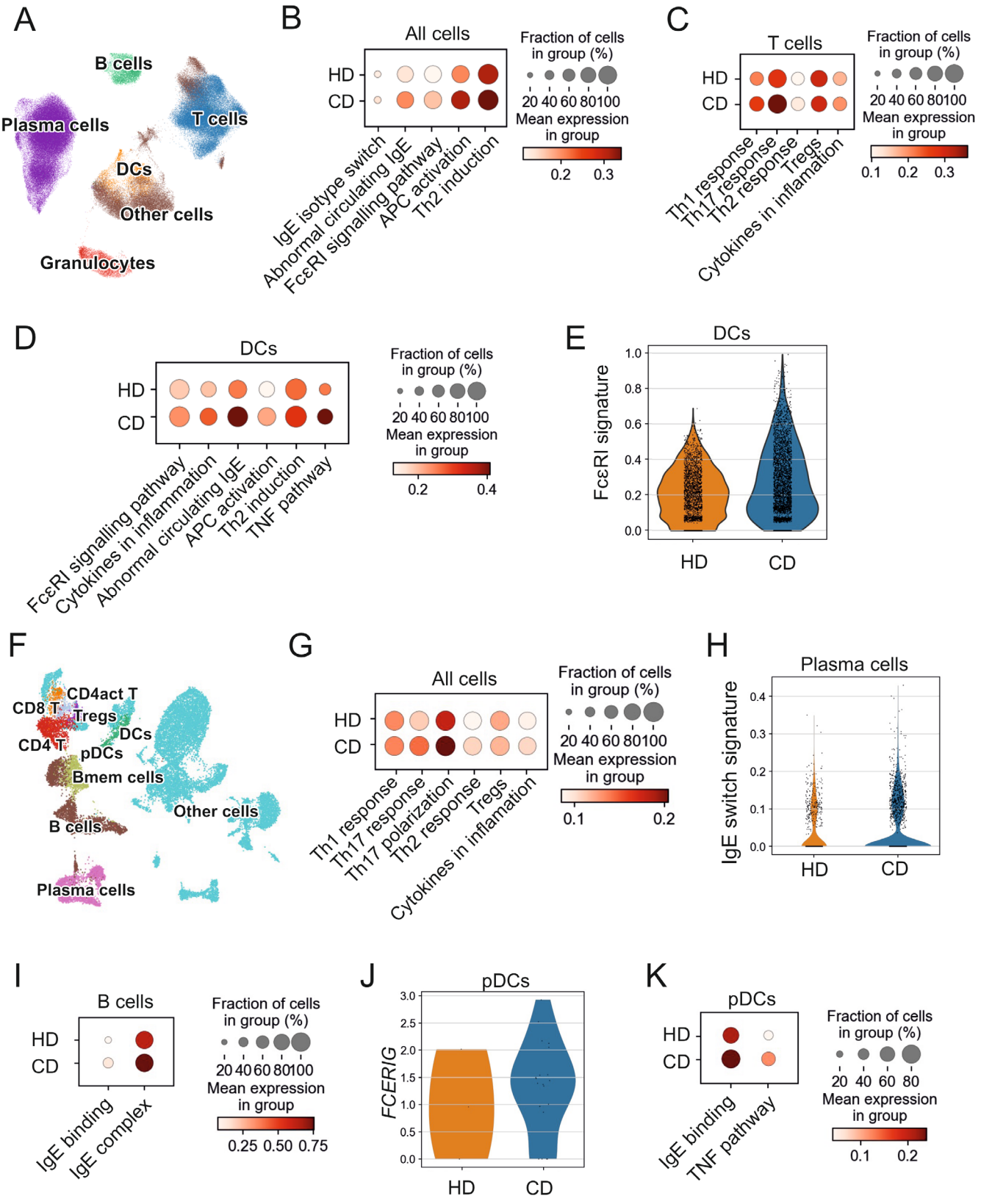

FIGURE 4 | Legend on next page.

isolated pDCs were incubated with or without omalizumab for 18 h (Figure 5A) and, as previously reported by our group, [27]. this treatment significantly reduced the levels of IgE bound to FcεRI on the surface of pDCs compared to untreated pDCs (from 65.6% ± 5.9% to 27.2% ± 4.2%) (Figure 5B). Next, we induced the IgE-FcεRIα cross-linking on pDCs, stimulated them with TLR9-ligand (TLR9-L) for 18 h, and co-cultured with allogeneic naïve $CD4^+$ T cells for 5 days (Figure 5A). As shown in Figure 5C,

FIGURE 4 | scRNAseq analysis uncover the potentiation of IgE-FcεRI signaling and imbalanced Th-responses in the gut of adults and children Crohn's disease patients. (A) UMAP plot illustrating clustering in adult gut single-cell data. (B) Expression score for the panel of IgE-related gene signatures across the whole adult single-cell dataset. (C) Expression score for T helper gene signatures across T cell cluster from adult dataset. (D) Expression score for the panel of IgE-related gene signatures in DC cluster from the adult dataset. (E) Violin plot depicting the expression score of FcεRI-related gene signature in DC from adult data. (F) UMAP plot displaying clustering in pediatric gut single-cell data. (G) Expression score for a panel of T helper gene signatures across the entire single-cell pediatric dataset. (H-I) Expression score for the IgE switch gene signature in plasma cell cluster (H) and IgE binding and IgE complex gene signatures in B cell cluster (I) within the pediatric dataset. (J-K) Expression of the FCERIG gene (J) and IgE binding and TNF-related gene signature score expression (K) in pDCs cluster. Dot plot: Size depicts % of expressing cells, color intensity encodes mean expression in the group.

IgE-FcεRIα cross-linking on pDCs significantly decreased the frequency of $CD4^+CD25^{high}CD127^-FOXP3^+$ Tregs generated by TLR9-L-activated pDCs from CD patients. Remarkably, pre-treatment of pDCs with omalizumab restored the capacity of TLR9-L-activated pDCs under IgE-FcεRIα cross-linking to induce Tregs (Figure 5C). Of note, all these in vitro assays were always performed in the presence of IL-3 to ensure pDC viability throughout the experiment. Collectively, these data show that IgE-FcεRIα cross-linking in TLR9-L-activated pDCs from CD patients impairs their capacity to generate Tregs, which is restored by pre-treatment of pDCs with omalizumab.

To further confirm our in vitro data, we analyzed cell–cell communication networks using scRNAseq datasets from gut samples of pediatric CD patients. As expected, pDCs and Tregs interactions were demonstrated in the intestine from both children with CD and HD (Figure 5D). The number of intercellular interactions between pDCs and Tregs, indicated by the weight of the interaction, was upregulated in HD compared to CD patients (Figure 5E). Different ligand-receptor pairs involved in the pDCs –Tregs interaction in the gut of CD patients were identified (Figure 5F), and gene ontology analysis uncovered an enrichment in pathways related to the regulation of activation, proliferation, and inflammation (Figure 5G). Overall, these data suggest that the pDCs –Tregs axis and therefore multiple processes involved in intestinal inflammation might be altered in CD patients compared to HD.

4 | Discussion

In this study, we uncover an unprecedented role for IgE in the pathophysiology of CD with potential relevant clinical implications. We show that CD patients included in this study display significantly higher frequencies of circulating IgE^+ or $FcεRIα^+$ immune cells than HD, reaching levels that are similar to those shown by allergic individuals without inflammatory bowel disease. Importantly, increased levels of IgE- and FcεRIα- bearing immune cells observed in patients with CD do not depend on their allergic status. We also demonstrate, for the first time, that CD patients display significantly higher numbers of circulating IgE^+ pDCs than HD, which is concomitant with lower numbers of $FOXP3^+$ Tregs and higher numbers of effector memory $CD4^+$ T cells. Supporting these data, detailed analysis of publicly available scRNAseq data from gut tissues confirms that overactivation of IgE-mediated pathways and Tregs imbalance also takes place in the intestines of both children and adults with CD. Proof of concept functional in vitro experiments demonstrate that IgE-FcεRIα cross-linking on pDCs from CD patients impairs their capacity to generate Tregs, which can be restored by omalizumab. Overall, our data indicate that the IgE-pDCs-Tregs axis might be altered in CD patients and connected to intestinal and systemic inflammation, which could represent a novel mechanism to be exploited for alternative therapeutic interventions.

Since the 1970s, the potential role of IgE in the pathophysiology of CD has been explored and discussed [14, 33, 34]. However, the specific link between IgE and the etiology or development of CD remains elusive. The association between food allergy and asthma with CD has been widely discussed since mechanisms partially overlap [7, 8, 14, 19, 35, 36]. Recently published observational studies reported a causal correlation between CD and asthma, but the potential molecular mechanisms underlying such an association remain to be discovered [9]. The interconnected relationship between the gut and the lung is commonly referred to as the gut-lung axis. Alterations in the composition of the gut microbiome, resulting from factors such as dietary changes, disease, or medical interventions like antibiotic use, are correlated with changes in immune responses and airway homeostasis [10, 11]. Therefore, there may be common unidentified molecular pathways underlying these diseases that could justify this reported causal correlation. Both asthma and CD are characterized by increased IgE levels [14, 37]. and it is plausible to speculate that IgE-mediated pathways, as they are very well established in asthma, could also contribute to promoting inflammation in CD. Therefore, in this study, we sought to further investigate the potential role of IgE and IgE-mediated signaling pathways within the context of CD. Fifty percent (10/20) of the CD patients included in our study also suffered from allergic diseases: most of them pollen-induced rhino-conjunctivitis, two were asthmatic, and none of them was diagnosed with food allergy. Remarkably, the allergic and non-allergic CD patients included in this study did not show significant differences in the frequency of circulating IgE^+ or $FcεRIα^+$ immune cells, indicating that such increased levels observed in CD patients compared to HD do not necessarily depend on the allergic status of CD patients. Similarly, a link between autoimmunity and CD has also been proposed, and CD is usually defined as an autoimmune disease [38]. Although there is emerging evidence for the role of IgE autoantibodies in many autoimmune diseases, the presence of self-reactive IgE in CD patients has not yet been identified [12, 39].

In this study, we performed a comprehensive characterization of different blood IgE^+ or $FcεRIα^+$ immune cell subsets to search for potential differences in functional specific IgE-mediated pathways between CD patients and HD beyond serum levels of total IgE. The frequencies of circulating IgE^+ or $FcεRIα^+$

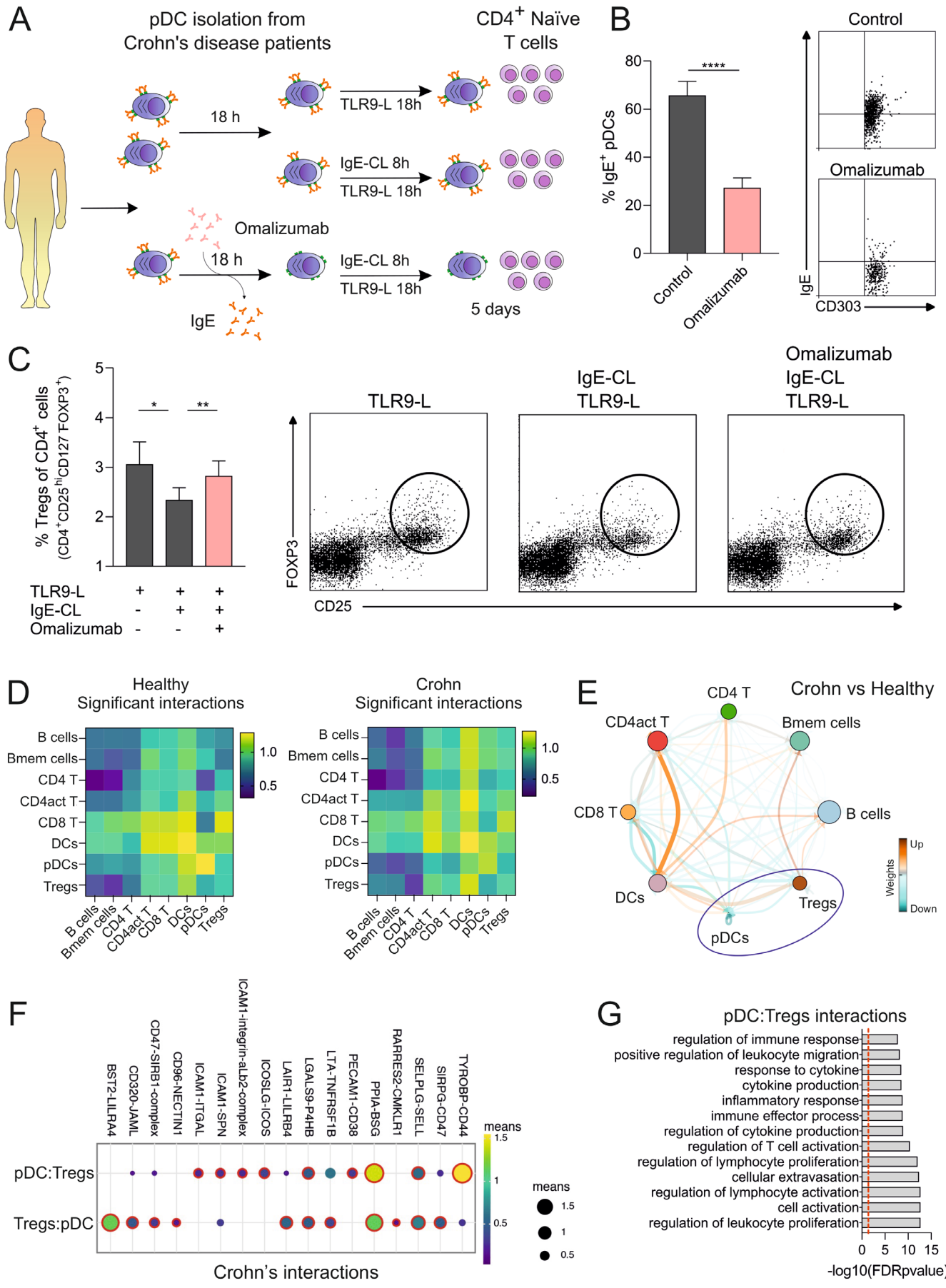

FIGURE 5 | Legend on next page.

FIGURE 5 | IgE-FcεRI cross-linking on pDCs from CD patients impairs the generation of Tregs. (A) Experimental outline. Freshly isolated pDCs from CD patients were incubated with or without omalizumab 10 mg/mL for 18 h. After several washes, pDCs were incubated for 8 h with IgE-crosslinker or its isotype control at 10 μg/mL. Then, pDCs were stimulated for 18 h with TLR9-L 2 μM. Finally, pDCs were cocultured for 5 days with allogeneic naïve CD4$^+$ T cells before analysis. (B) Effect of omalizumab incubation on the percentage of IgE$^+$ pDCs isolated from CD patients. (C) Percentages of CD4$^+$CD25^{high}CD127$^-$FOXP3$^+$ Tregs induced by pDCs from CD patients (gating in lymphocytes) ($n = 17$–20). Paired Student's t test, $^*p < 0.05$, $^{**}p < 0.01$ and $^{****}p < 0.0001$. (D) Heatmap showing the total number of interactions with significant means between cell types in the gut dataset from pediatric CD patients and HD obtained with CellPhoneDB. (E) Comparative crosstalk analysis of ligand-receptor interactions between immune cell populations from intestine of CD patients versus HD. (F) Overview of ligand–receptor interactions between pDCs and Tregs in the gut of CD pediatric patients; P values indicated by circle size, scale on right. The means of the average expression level of interacting molecule 1 in pDC cluster and interacting molecule 2 in Tregs cluster are indicated by color. Significant interactions are marked with red circle. (G) Gene ontology for the ligands and receptor molecules implicated in cell–cell communication between pDCs and Tregs in CD pediatric patients.

basophils were significantly higher in CD patients than in HD. These data suggest that IgE-mediated degranulation of basophils and the release of inflammatory mediators could also contribute to mucosal inflammation in CD, which is aligned with previous studies investigating the role of mast cells and basophils in IBD [34, 40, 41]. Similarly, the elevated frequencies of IgE$^+$ mDCs 2 in CD patients suggest their potential involvement in chronic inflammation by activating inflammatory memory Th2 cells via mechanisms depending on IgE-mediated presentation [42]. Of note, CD patients show higher frequencies of IgE$^+$ or FcεRIα$^+$ pDCs than HD, and gut-resident pDCs from CD patients show an increased expression of *FCERIG* and genes related to IgE-binding compared to HD. Supporting these data, higher frequencies of proinflammatory pDCs in the mucosal space and mesenteric lymph nodes of IBD patients have been previously reported [43]. Interestingly, herein, we observed that in addition to higher levels of IgE$^+$ pDCs, CD patients also display a significant reduction of circulating Tregs and naïve T cells and increased levels of effector T cells compared to HD, which would contribute to the activation of other proinflammatory cells such as macrophages, monocytes, or endothelial cells [25, 44]. It is well described that CD patients display excessive Th1 and Th17 responses [44]. Accordingly, gene signature analyses performed in intestinal samples of CD patients showed higher Th1 and Th17 responses compared to healthy guts. Although we did not analyze the frequencies of these CD4$^+$ T cell subset lineages in the cohorts of patients included in this study, it is tentative to speculate that the observed increased frequencies of effector memory CD4$^+$ T cells found in CD patients might be enriched in Th1 and Th17 cells. Functional Tregs are crucial in regulating immune responses by promoting immune tolerance and controlling the onset and progression of inflammatory and autoimmune diseases such as asthma or CD [45, 46]. In the context of asthma, we previously showed a link between IgE-FcεRI cross-linking on human pDCs and Tregs impariment [27, 28]. Of note, in this study our functional in vitro experiments demonstrate that IgE-FcεRI cross-linking on pDCs also impairs their capacity to generate Tregs in CD patients, which is restored by omalizumab. Collectively, these data suggest that, as reported for other diseases, the IgE-pDCs-Tregs axis might also be altered in CD patients, thus likely contributing, in cooperation with other well-described mechanisms, to the local and systemic inflammation associated with CD. The analysis of scRNAseq data shows that the number of specific ligand-receptor interactions between pDCs and Tregs is significantly decreased in CD patients compared to HD. Collectively, all this data indicate that the axis IgE-pDCs-Tregs might well also contribute to the pathophysiology of CD by impairing the generation of functional Tregs and promoting inflammatory responses.

The anti-IgE mAb omalizumab is approved for several IgE-mediated diseases, such as allergic asthma, chronic spontaneous urticaria (CSU), and chronic rhinosinusitis with nasal polyps (CRSwNP) [47]. In addition, very recently, the FDA approved omalizumab for the treatment of IgE-mediated food allergy [48]. As discussed above omalizumab restores the capacity of pDCs from CD patients to generate Tregs, representing the first evidence to suggest a potential use of omalizumab in CD. Our data also demonstrate higher levels of surface-bound IgE on basophils, which are important immune cells associated with the mode of action of omalizumab [49–51]. In this regard, IBD mouse models have previously shown that IgE blockage protects from the onset of colitis by modulating the functions of basophils and mast cells [52, 53]. Therefore, by also targeting these cells, omalizumab could induce beneficial effects in CD patients beyond the restoration of Tregs. Currently, the data related to the use of omalizumab in CD is very limited, and only a few case reports in which CD was concomitant with CSU have been reported [54, 55].

In summary, we uncover unprecedented molecular mechanisms by which IgE could impair Treg function in the context of CD, which might contribute, in cooperation with other well-described mechanisms, to promote and amplify inflammation in CD. Our findings provide evidence of the potential involvement of the IgE-pDCs-Tregs axis in the regulation of inflammatory responses in CD. Of note, this novel pathway in CD could also represent a shared immunological mechanism underlying the causal relationship between CD and asthma in those patients in whom both diseases coexist, a possibility that still needs further research. Personalized medicine stands as one of the main goals for the treatment of CD, [56]. not only to control the symptoms but also to prevent disability in the long term [3]. The better understanding of the molecular mechanism underlying CD and the potential role played by IgE and IgE-mediated pathways might well pave the way for the development of alternative therapeutic interventions.

Author Contributions

Conceived and designed the study: O.P. Performed all the in vitro experiments and analyzed the data: A.R.-M., and C.B.-V. Performed scRNAseq analysis: S.S. Clinical diagnosis and management of patients

and samples: C.T., D.O., M.A.G.-B., and J.C. Provided reagents, protocols, and materials: A.A., L.M.-C., P.T., X.J., C.T., and O.P. Interpreted and discussed the data: A.R.-M., C.B.-V., D.O., S.S., M.A.G.-B., A.A., L.M.-C., J.C., P.T., X.J., C.T., and O.P. Wrote the paper: O.P., A.R.-M., and C.B.-V. All the authors read and approved the final version of the manuscript.

Conflicts of Interest

O.P. received research grants from MINECO, Ministerio de Ciencia e Innovación, CAM, Inmunotek S.L., Novartis, and AstraZeneca, and fees for giving scientific lectures or participation in Advisory Boards fromAstraZeneca, Pfizer, GlaxoSmithKline, Inmunotek S.L., Novartis, Sanofi-Genzyme, and Regeneron. P.T. and X.J. are Novartis employees. C.T. has served as a speaker, consultant, and advisory board member for MSD, AbbVie, Pfizer, Takeda, Janssen, Galapagos, Lilly, Fresenius Kabi, Ferring, Faes Farma, Shire Pharmaceuticals, Dr. Falk Pharma, and Tillots. The rest of the authors declare no competing financial interests.

Data Availability Statement

All data are available and will be provided by the authors upon reasonable request. Gene set enrichment signatures were analysed from available single-cell(sc)RNAseq datasets for adults (SCP1884) and children (E-MTAB-8901) Crohn's disease patients through CZ CELLxGENE Discover.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.