Clinical Immunology 260 (2024) 109923

Contents lists available at ScienceDirect

Clinical Immunology

journal homepage: www.elsevier.com/locate/yclim

IL-10-producing regulatory cells impact on celiac disease evolution

Laura Passerini [a,1], Giada Amodio [a,1], Virginia Bassi [a], Serena Vitale [b], Ilaria Mottola [b], Marina Di Stefano [c], Lorella Fanti [d], Paola Sgaramella [c], Chiara Ziparo [e], Silvia Furio [e], Renata Auricchio [f], Graziano Barera [c], Giovanni Di Nardo [e], Riccardo Troncone [f], Carmen Gianfrani [b,2], Silvia Gregori [a,*,2]

[a] Mechanisms of Peripheral Tolerance Unit, San Raffaele Telethon Institute for Gene Therapy (SR-Tiget), IRCCS San Raffaele Scientific Institute, Via Olgettina 60, Milan 20132, Italy
[b] Institute of Biochemistry and Cell Biology, CNR, Via P. Castellino 111, Naples 80131, Italy
[c] Department of Paediatrics, IRCCS San Raffaele Scientific Institute, Via Olgettina 60, Milan 20132, Italy
[d] Gastroenterology and Gastrointestinal Endoscopy Unit, IRCCS San Raffaele Scientific Institute, Via Olgettina 60, Milan 20132, Italy
[e] NESMOS Department, School of Medicine and Psychology, Sapienza University of Rome, Sant' Andrea University Hospital, Via di Grottarossa 1035, Rome 00189, Italy
[f] European Laboratory for the Investigation of Food Induced Diseases (ELFID), Department of Translational Medical Science, Section of Pediatrics, Via Pansini 5, University Federico II, Naples 80131, Italy

ARTICLE INFO

Keywords:
Interleukin 10
Potential celiac disease
Tolerogenic dendritic cells
T regulatory type 1
Gliadin-specific T cells
Villous atrophy

ABSTRACT

Celiac Disease (CD) is a T-cell mediated disorder caused by immune response to gluten, although the mechanisms underlying CD progression are still elusive. We analyzed immune cell composition, plasma cytokines, and gliadin-specific T-cell responses in patients with positive serology and normal intestinal mucosa (potential-CD) or villous atrophy (acute-CD), and after gluten-free diet (GFD). We found: an inflammatory signature and the presence of circulating gliadin-specific IFN-γ^+ T cells in CD patients regardless of mucosal damage; an increased frequency of IL-10-secreting dendritic cells (DC-10) in the gut and of circulating gliadin-specific IL-10-secreting T cells in potential-CD; IL-10 inhibition increased IFN-γ secretion by gliadin-specific intestinal T cells from acute- and potential-CD. On GFD, inflammatory cytokines normalized, while IL-10-producing T cells accumulated in the gut. We show that IL-10-producing cells are fundamental in controlling pathological T-cell responses to gluten: DC-10 protect the intestinal mucosa from damage and represent a marker of potential-CD.

1. Introduction

Celiac disease (CD) is T-cell a mediated disease triggered by gluten in genetically susceptible individuals [1,2], who develop a permanent gluten intolerance with production of anti-tissue transglutaminase (tTG) and anti-endomysium antibodies and a marked T-cell response to gliadin, a gluten component. Small intestinal intra-epithelial and lamina propria T lymphocytes intervene in the inflammatory cascade that leads to mucosa damage [3,4]. Although 30% of Europeans carry the HLA risk alleles and gluten-containing cereals are largely consumed, only 1% of subjects develop CD. Moreover, 10% of genetically predisposed subjects with positive serology and normal intestinal mucosa are classified as potential-CD, and only 30% of them develop overt-disease in 9 years [5,6].

The pivotal role of IL-10 in controlling intestinal inflammation in CD is supported by studies in mice with IL-10-deficient signaling in antigen-presenting cells (APCs): mice develop spontaneous intestinal inflammation [7] and crypt hyperplasia upon gluten challenge [8], indicating that IL-10-signaling is crucial to prevent gluten-induced intestinal inflammation. In TCR/HLA-DQ2-transgenic mice ingestion of

Abbreviations: APC, Antigen presenting cells; CD, Celiac Disease; DC, Dendritic cells; DC-10, IL-10-producing dendritic cells; EBV, Epstein Bar Virus; EGDS, Esophagogastroduodenoscopy; GFD, Gluten free diet; PB, Peripheral blood; PT-TG, Deamidated peptic-tryptic digest of gliadin; SFU, Spot forming unit; Tr1, T regulatory type 1; tTG, Tissue transglutaminase..

* Corresponding author at: Mechanisms of Peripheral Tolerance Unit, San Raffaele Telethon Institute for Gene Therapy (SR-Tiget), IRCCS San Raffaele Scientific Institute, Via Olgettina 60, Milan 20132, Italy.
E-mail address: gregori.silvia@hsr.it (S. Gregori).

[1] These authors contributed equally to this work and share first authorship.
[2] These authors contributed equally to this work and share senior authorship.

https://doi.org/10.1016/j.clim.2024.109923
Received 13 October 2023; Accepted 1 February 2024
Available online 4 February 2024

deamidated-gliadin induced expansion of IFN-γ and IL-10-producing T cells, with the latter cells suppressing gliadin hypersensitivity in sensitized recipients [9]. In a transfer model of CD, treatment with gliadin PLG-nanoparticles protected mice from enteropathy by increasing gliadin-specific IL-10 production [10]. In humans, IL-10 gene polymorphisms are associated to CD [11–13] and several reports suggest that an unbalance between IL-10 and IFN-γ occurs in damaged intestinal mucosa [14,15]. Moreover, gliadin-specific pro-inflammatory T-cell response was described at early manifestation of acute-CD [16], while gliadin-specific IFN-γ production by intestinal T cells from potential-CD children increased upon blocking of IL-10/TGF-β [17].

Gluten-specific IL-10-producing T regulatory type 1 (Tr1) clones have been isolated from the small intestinal mucosa of CD patients on gluten-free diet (GFD-CD) [18] and IL-10 expression was upregulated in potential-CD mucosa [14]. While these data support the pivotal role of Tr1 cells in CD, little is known on their role in the natural CD history, as well as of IL-10-producing APCs in controlling gut inflammation. Here, we characterized the immune cell compartments in peripheral blood (PB) and in gut mucosa infiltrates, and quantified cytokines in the plasma of CD patients at different stage of disease (potential-CD, acute-CD, GFD-CD). We show that while a pro-inflammatory environment prevails without GFD, the presence of a peculiar subset of IL-10-producing dendritic cells (DC-10) [19] uniquely characterizes the intestinal mucosa of potential-CD patients. We confirm an enrichment of Tr1 cells in the mucosa after GFD. Our results support the previously described role of IL-10 in regulating CD evolution and identify the IL-10-producing DC as a novel target for CD therapy.

2. Material and methods

2.1. Study population

CD patients enrolled in the study were classified as: acute-CD, with villous atrophy of small intestinal mucosa (Marsh 3); potential-CD, with normal/not-inflamed mucosa (Marsh 0), or mucosa with a mild lymphocyte infiltration (Marsh 1); GFD-CD, on gluten free diet since at least 6 months. Overall, the study population includes 90 pediatric CD patients (33 potential-CD, 45 acute-CD and 12 CD patients on GFD (GFD-CD) (Tables E1-E3, Table 1), and 24 age-matched non-CD controls ($n = 9$ who underwent duodenal biopsy, Table S4, and $n = 15$ for PB samples, protocol TIGET09). Subjects were enrolled at the San Raffaele Scientific Institute of Milan, Sant' Andrea University Hospital of Rome, and at the University Federico II of Naples. PB and intestinal biopsies were collected from subjects undergoing esophagogastroduodenoscopy (EGDS) for CD diagnosis or follow up, upon signature of the informed consent in accordance with the Helsinki Declaration and with local ethical committee approvals (protocol TIGET12b; protocol Federico II 308/16). Participants of the non-CD group underwent EGDS as follow up for caustic injestion or suspect of CD, disproved by histological workout. Acute-CD and potential-CD patients had, at the time of diagnosis, positive serology of CD-autoantibodies (positive threshold anti-tTG Abs IgA > 10 U/ml, IgA Endomysial antibody (EMA)).

2.2. Intestinal cell isolation

Small intestinal duodenal biopsies were digested for 1.5 h at 37 °C with 1.5 mg/ml of collagenase-A from *Clostridium histolyticum* (Roche, Basel, Switzerland), as previously described [20]. Depending on size of the bioptic fragment and degree of infiltration, the cellular yield was variable (alive cell count: 0.2–1.5 × 10^6). Samples with low (<0.6 × 10^6), mid (0.6–1 × 10^6), and high (>1 × 10^6) cell yield were used to analyze lymphoid, myeloid, or both infiltrates, respectively.

2.3. Generation of gliadin-specific T-cell lines

T-cell lines were generated from cells isolated from small intestinal biopsies (Table 1 and supplementary materials), as previously described [21]. Briefly, 3–5 × 10^5 cells/24-well were stimulated with 1.5 × 10^6 irradiated (35Gy) autologous PBMC in the presence of 50 μg/ml deamidated peptic-tryptic digest of gliadin (PT-TG), and 50 U/ml IL-2 and 5 ng/ml IL-15 (R&D System Minneapolis, MN) was added every 3 days. After a second PT-TG stimulation, T cells were stimulated for 48 h with autologous or HLA-matched Epstein-Barr Virus (EBV) immortalized B-cells (1 × 10^5) and PT-TG (50 μg/ml), with or without 10 μg/ml of anti-IL-10R (BD Biosciences, Franklin Lakes, NJ) and anti-TGF-β1,2,3 Abs (R&D System). IFN-γ production was measured in supernatants by ELISA (Mabtech, Nacka Strand, Sweden).

2.4. Flow cytometry

The frequencies of cell subsets in PB were assessed by flow cytometry, as previously described [22]. Dead cell exclusion was performed using Live/Dead PromoFluor 840 Maleimide reactive dye (Invitrogen, Carlsbad, CA). Samples were fixed in 0.5% paraformaldehyde (PFA, Thermo Fisher Scientific, Whaltam, MA). Cell suspensions from small intestinal biopsies were stained with PromoFluor 840 Maleimide reactive dye (Invitrogen), washed, resuspended in staining buffer plus FcR-Blocking reagent (Miltenyi Biotech, Bergish Gladbach, Germany)

Table 1
Clinical characteristics of patients enrolled in the study from which we generated intestinal T-cell lines.

Patient Code	Diagnosis	Age (ys.m)	Sex	anti-tTG IgA (U/ml)	EMA	HLA-DQ alleles	Marsh –Oberhuber Score
CD 1	Acute-CD	15.3	F	145	Pos	2.2/8	Marsh 3a
CD 4	Acute-CD	13.1	M	2371.5	Pos	2.2	Marsh 3c
CD 5	Acute-CD	10.1	F	20	Pos	8	Marsh 3c
CD 6	Acute-CD	13.8	F	25	Neg	2.5	Marsh 3c
CD 7	Acute-CD	4.3	M	58.4	Pos	2.5	Marsh 3b/c
N = 5		Mean (range) 11.3 (4.3–15.3)	F/M 3/2	Mean (range) 524.0 (20–2371.5)	Pos/Neg 4/1		Marsh 3a/3b-c 1/4
Patient Code	**Diagnosis**	**Age (ys.m)**	**Sex**	**anti-tTG IgA (U/ml)**	**EMA**	**HLA-DQ alleles**	**Marsh –Oberhuber Score**
POT 2	Potential-CD	2.1	F	29	Neg	8	Marsh 0
POT 4	Potential-CD	14.1	F	23	weak Pos	2.5	Marsh 1
POT 7	Potential-CD	2.1	F	18.6	Pos	2.5	Marsh 1
POT 8	Potential-CD	2.3	F	100	Pos	2.5	Marsh 1
POT 9	Potential-CD	2.5	F	41	Pos	2.5	Marsh 1
N = 5		Mean (range) 4.7 (2.1–14.1)	F/M 5/0	Mean (range) 42.3 (18.6–100)	Pos/Neg 4/1		Marsh 0/1 1/4

Anti-tTG: anti-tissue transglutaminase antibodies, cut-off for positive serology: > 7 U/ml by immunoenzymatic assay; EMA: Endomysial autoantibodies; F: female; M: male; NA: not available; Neg: negative; Pos: positive; HLA-DQ2.2: DQA1*0201$^+$DQB1*02$^+$; HLA-DQ2.5: DQA1*05$^+$DQB1*02$^+$; HLA-DQ8: DQB1*0302$^+$; HLA-DQ7: DQA1*05$^+$DQB1*.

containing Abs, and incubated for 15 min at RT in the dark. Intranuclear Foxp3 staining was performed on cell suspensions using FOXP3 transcription factor buffer set, following manufacturer's instructions (eBioscience, San Diego, CA). For Tr1 cell detection, cells were stained as previously described [23]. Abs used are listed in Table S5.

For consistency of results, only bioptic samples with $CD45^+$ cell counts >10,000 were considered for analysis.

Samples were acquired using CytoFLEX-LX (Beckman Coulter, Jersey City, NJ) analyzer and data analyzed using FlowJo v10 (Ashland, OR) or FCS express v6 (*De Novo* Software, Pasadena, CA) softwares. Quadrant markers were set according to unstained controls.

2.5. Cytokine determination

Peripheral plasma of CD patients and HC were collected and stored at ≤20 °C. Samples were centrifuged at 1000 ×*g* for 15 min immediately prior to use. The presence of: IL-1β, TNF-α, IL-6, IL-8, IL-10, IL-12p70, IFN-γ, IL-10, IL-2, IL-4, IL-15, and IL-17 was evaluated by magnetic bead-based multiplex assay (R&D Systems) using MAGPIX System (Luminex, Austin, TX), according to manufacturer's instructions.

2.6. ELISPOT assays

2×10^5 PBMC were plated onto ELISPOT plates (Millipore, Burlington, MA) pre-coated with anti-IFN-γ (10 μg/ml, Mabtech, clone 1-D1K) or anti-IL-10 (10 μg/ml, Mabtech clone 9D7) capture Abs in X-VIVO15 medium (Lonza, Verviers, Belgium) supplemented with 5% human serum (Lonza) and penicillin/streptomycin (Euroclone) alone or with the gliadin peptide (DQ2.5-glia-α1,2 QLQPFPQPELPYPQPQP, hereafter glia-peptide). After 42–48 h, IFN-γ- or IL-10-producing cells were detected by biotin-conjugated anti-IFN-γ (1 μg/ml, Mabtech clone 7-B6–1) or anti-IL-10 (1 μg/ml, Mabtech clone12G8) Abs, followed by streptavidin-peroxidase (Roche) and reaction with 3-Amino-9-ethylcarbazole substrate (BD Biosciences). Spots were counted by ImmunoSpot-S6-ultraV (Cellular Technology Limited, Shaker Heights, OH, USA). A peptide pool (PepTivator® CMV pp65, Miltenyi Biotec) and phytohemagglutinin (PHA, Sigma-Aldrich) were used as controls. Results were normalized to spot forming units (SFU)/10^6 PBMC. Patients were considered responders (R) if the absolute SFUs in response to -glia 17mer exceeded the SFUs count of negative control peptide wells.

2.7. HLA-DQ typing

For the assessment of HLA-DQ haplotypes, DNA was extracted from PB by QiAMP DNA-Blood-Mini kits (Qiagen, Hilden, Germany), and the presence of the alleles DQB1*0302 (HLADQ8), DQA1*05/DQB1*02 (HLADQ2.5), DQA1*0201/DQB1*02 (HLADQ2.2), DQA1*05/DQB1*0301 (HLADQ7) was investigated by PCR (EuGen Kit, Eurospital, Trieste, Italy), following the manufacturer's instructions.

2.8. Statistical analysis

Data were analyzed using Prism 8.0 (GraphPad-Prism, La Jolla, CA). For comparisons between two groups, non-parametric Mann-Whitney U or Wilcoxon signed-rank tests were used for unpaired or paired comparisons, respectively. For multiple independent groups comparisons, Kruskall-Wallis test with Benjamini-Hochberg FDR correction was applied. $p \leq 0.05$ was regarded as significant. Inferential analyses were applied in the presence of $n \geq 5$, otherwise only descriptive statistics are reported.

3. Results

3.1. CD patients display a pro-inflammatory signature in the periphery regardless of disease stage

In the hypothesis that IL-10-producing cells are involved in maintaining tolerance in CD, we investigated the presence of DC-10 and Tr1 cells in the PB of potential-CD, acute-CD (Tables S1-S2) and healthy children. No significant differences in the frequency of CD3, CD19, CD56, CD14, CD15, CD4, CD8, and TCRγδ T cells were observed among the different groups (Fig. S1). The frequency of DC-10 ($CD11c^+CD14^+CD16^+CD163^+CD141^+$) in potential-CD, acute-CD, and HC subjects was comparable, with only few potential-CD patients showing a higher DC-10 frequency than HC. cDC1 ($CD14^-CD11c^+CD141^+$) and pDC ($CD11c^-CD303^+$) frequencies were comparable, whereas we found a high proportion of cDC2 ($CD11c^+CD1c^+$) in a fraction of potential-CD and acute-CD patients (Fig. 1A). Tr1 and $FOXP3^+$Treg cell frequencies were similar in patients and controls, with only few acute-CD patients showing a higher Tr1 cell frequency than HC (Fig. 1B). While IL-12p70 and IFN-γ plasma levels were undetectable/barely detectable in HC, high levels of these Th1 cytokines were found in a subset of potential-CD and acute-CD patients. Few patients also displayed peaks of IL-1β and IL-8. No differences in TNF-α and IL-10 plasma levels were observed (Fig. 1C). Furthermore, higher proportion of circulating $CD4^+Ki67^+$ T cells were detected in potential-CD compared to acute-CD patients (Fig. 1D).Overall, a peripheral pro-inflammatory milieu, featured by inflammatory DC and activated T cells and increased Th1 cytokines, characterizes CD patients at potential and active stage of disease, thus suggesting that inflammation is likely related to gluten intake, rather than to the degree of gut-mucosal damage.

3.2. Increased gliadin-specific IL-10 response in the peripheral blood of potential-CD patients

We detected gliadin-specific T cells (IFN-γ^+ and/or IL-10^+) in 8/13 (61.5%) acute-CD and 7/9 (77.8%) potential-CD patients (Fig. 2A). Despite the high variability, glia-peptide stimulation induced IFN-γ-secreting T cells in acute-CD (5/6, 83.3%) and potential-CD (4/6, 66.7%) patients (Fig. 2B-C). Interestingly, T cells from most potential-CD patients responsive to gliadin released IL-10 upon glia-peptide stimulation (5/7, 71.4%), while only half of the acute-CD patients (4/8) secreted IL-10 upon stimulation (Fig. 2B-C-D). Variable response to CMV-pp65-derived peptides was detected in all subjects, likely depending on age and previous exposure to the pathogen, and no specific skew of the response in any group (Table S6).

These data confirm a Th1-prone environment in CD patients [24], although circulating gliadin-specific IFN-γ-producing T cells are detectable at low frequency. Interestingly, gliadin-specific IL-10-producing T cells are also present in the PB of patients, but they are more frequently found in potential-CD subjects.

3.3. Tolerogenic DC-10 are present in the gut mucosa of potential-CD patients

We then characterized immune cells infiltrating the gut mucosa of potential-CD and acute-CD patients. Subjects with negative serology, not inflamed mucosa, and unspecific histology served as non-CD controls (Table S4). According to the degree of tissue damage (Tables E1-E2), we detected a significantly increased percentage of $CD45^+$ cells infiltrating the mucosa of acute-CD, compared to both potential-CD and non-CD subjects (Fig. 3A). The composition of the infiltrate showed prevalence of $CD3^+$ T cells, with a significant increase in acute-CD compared to potential-CD patients. B lymphocytes ($CD19^+$) were also highly represented in the intestinal infiltrate and constituted a distinctive trait of acute-CD patients (Fig. 3B-C). As previously reported [20,25,26], we

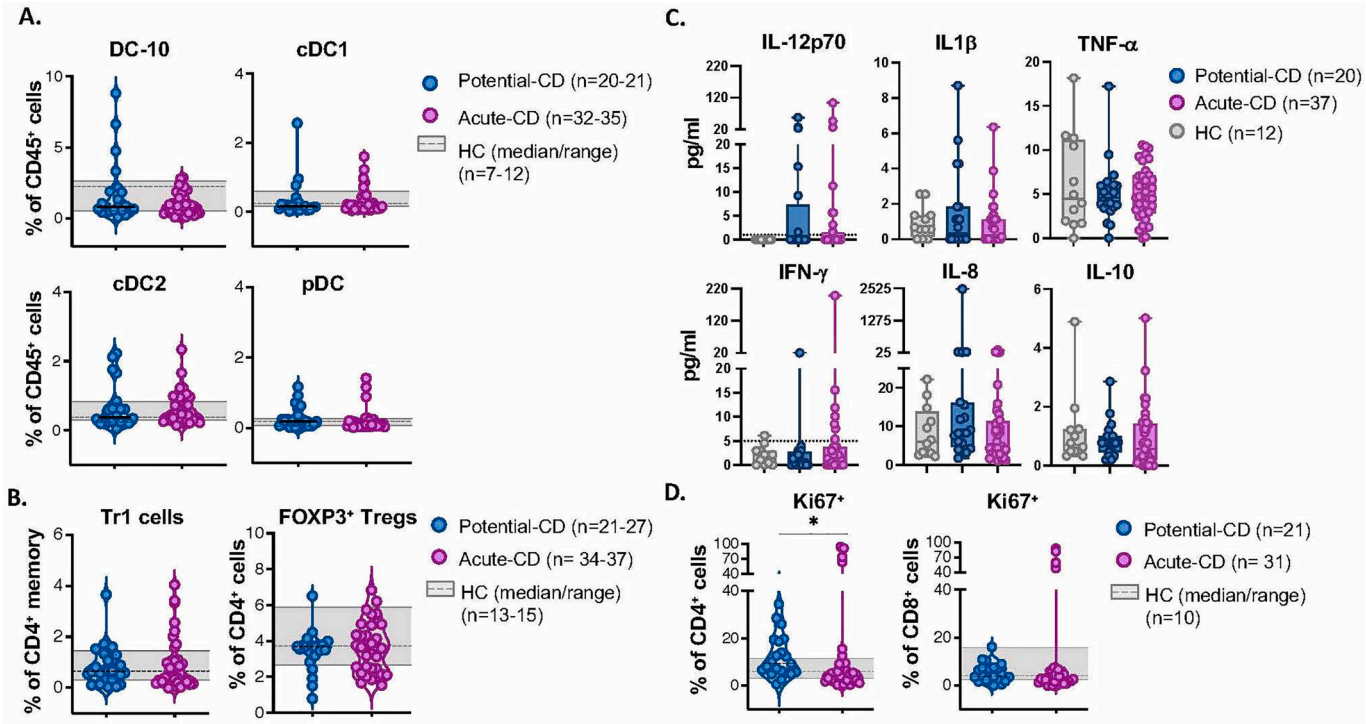

Fig. 1. Pro-inflammatory signature in peripheral blood of untreated CD patients. The proportion of the major leukocyte subsets in the peripheral blood of potential-CD patients, CD patients with atrophic gut mucosa (acute-CD), and age-matched healthy controls (HC) was analyzed by flow cytometry. (A) The frequencies of DC-10 ($CD11c^+CD14^+CD16^+CD141^+CD163^+$), cDC1 ($CD14^-CD11c^+CD141^+$), cDC2 ($CD11c^+CD1c^+$), and plasmacytoid (pDC, $CD11c^+CD303^+$), (B) of Tr1 cells ($CD49b^+LAG\text{-}3^+$) within the memory $CD4^+$ T cell compartment, and of $FOXP3^+$ Tregs ($CD127^-CD25^+FOXP3^+$) within the $CD4^+$ T cell compartment of potential-CD and acute-CD patients is plotted in the graphs. Range HC =25th–75th percentile (shaded area). Violin plots are shown, dots represent single patients. (C) The concentration of IL-12p70, IL-1β, TNF-α, IFN-γ, IL-8, and IL-10 in the plasma of potential-CD, acute-CD, and age-matched HC was evaluated using multiplex bead array assays. Box and whiskers (min to max) are shown, dots represent single donors. (D) The frequencies of $Ki67^+$ T cells within the $CD4^+$ (left panel) and $CD8^+$ (right panel) T cell compartment of potential-CD and acute-CD patients is plotted in the graphs. Range HC =25th–75th percentile (shaded area). Violin plots are shown, dots represent single patients. Kruskall-Wallis test with Benjamini-Hochberg FDR correction was applied. *: potential-CD *vs* acute-CD patients. $P \leq 0.05$.

observed a higher proportion of $TCR\gamma\delta^+$ T cells in acute-CD compared to non-CD gut-mucosa, although the combined comparison among the three groups did not reach statistical significance (Fig. 3C). No differences in the frequency of $CD4^+$ T cells were observed, regardless of the degree of tissue damage degree, while a reduced proportion of $CD8^+$ T cells was found in CD patients compared to non-CD subjects (Fig. 3C). The expression of CD25 and LAG-3 in T cells was comparable in patients and controls, while we detected high frequency of proliferating ($Ki67^+$) $CD8^+$ T cells in 60% of acute-CD patients (Fig. S2).

Tr1 cell frequency was comparable in potential-CD, acute-CD, and non-CD subjects, while the percentage of $FOXP3^+$Tregs was significantly lower in potential-CD than in acute-CD patients (Fig. 3D).

Analysis of the myeloid compartment showed comparable percentage of $CD11c^+$ cells infiltrating the small intestinal mucosa of CD patients and a significantly lower frequency of $CD14^+$ cells in potential-CD than in acute-CD patients (Fig. 4A-B). Interestingly, the frequency of DC-10 in potential-CD mucosa was higher compared to the acute-CD mucosa (Fig. 4C). pDC were the most abundant sub-population and their frequency was similar in acute-CD and potential-CD patients. cDC1 and cDC2 were present in CD gut-mucosa regardless of the degree of tissue damage (Fig. 4C), as previously reported [27].

3.4. Tr1 cells control the inflammatory response to gluten in CD gut mucosa

T-cell lines generated from gut-biopsies of CD patients (Table 1) produced IFN-γ in 5/5 potential-CD and in 2/5 acute-CD patients when restimulated with deamidated peptic-tryptic digest of gliadin (PT-TG) (Fig. 5A), confirming that gliadin-specific T cells infiltrate the mucosa of CD patients [17,28,29]. Interestingly, in the presence of anti-IL-10R/anti-TGF-β Abs, both acute-CD and potential-CD T-cell lines produced more IFN-γ in response to gliadin (Fig. 5B).

Overall, alterations in lymphoid and myeloid compartments and gliadin-specific IFN-γ-secreting T cells are present in the mucosa of all CD patients, regardless of tissue damage. The increase of IFN-γ from intestinal T-cell lines upon inhibition of IL-10/TGF-β pathways supports the presence of gliadin-specific Tr1-like cells in the intestine of CD patients. The prevalence of DC-10 exclusively in the intestinal mucosa of potential-CD subjects suggests that these cells could contribute in maintaining tissue homeostasis and controlling pathogenic T-cell responses.

3.5. Tr1 cells accumulate in the mucosa of CD patients on gluten free diet

We next investigated how removal of gluten from diet shapes the inflammatory infiltrate in the gut mucosa and impacts on the peripheral inflammatory signature of CD patients. According to the degree of tissue damage (Table S3), the percentage of $CD45^+$ cells in the gut mucosa was significantly lower in GFD-CD compared to acute-CD patients (Fig. 6A). This reduction was paralleled by changes in the immune cell infiltrate composition, with overall similar proportion of lymphoid cells, but significantly higher frequency of $TCR\text{-}\gamma\delta^+$ T cells in GFD-CD compared to acute-CD (Fig. 6B). These data confirm that aberrant accumulation of innate lymphoid cells in CD gut persists upon GFD regimen [30]. Furthermore, we confirmed the presence of Tr1 cells in biopsies from GFD-CD patients [18], with a significantly higher proportion compared to acute-CD patients (Fig. 6C). Conversely, no difference in $FOXP3^+$ Treg frequency was found (Fig. 6C). GFD did not induce significant

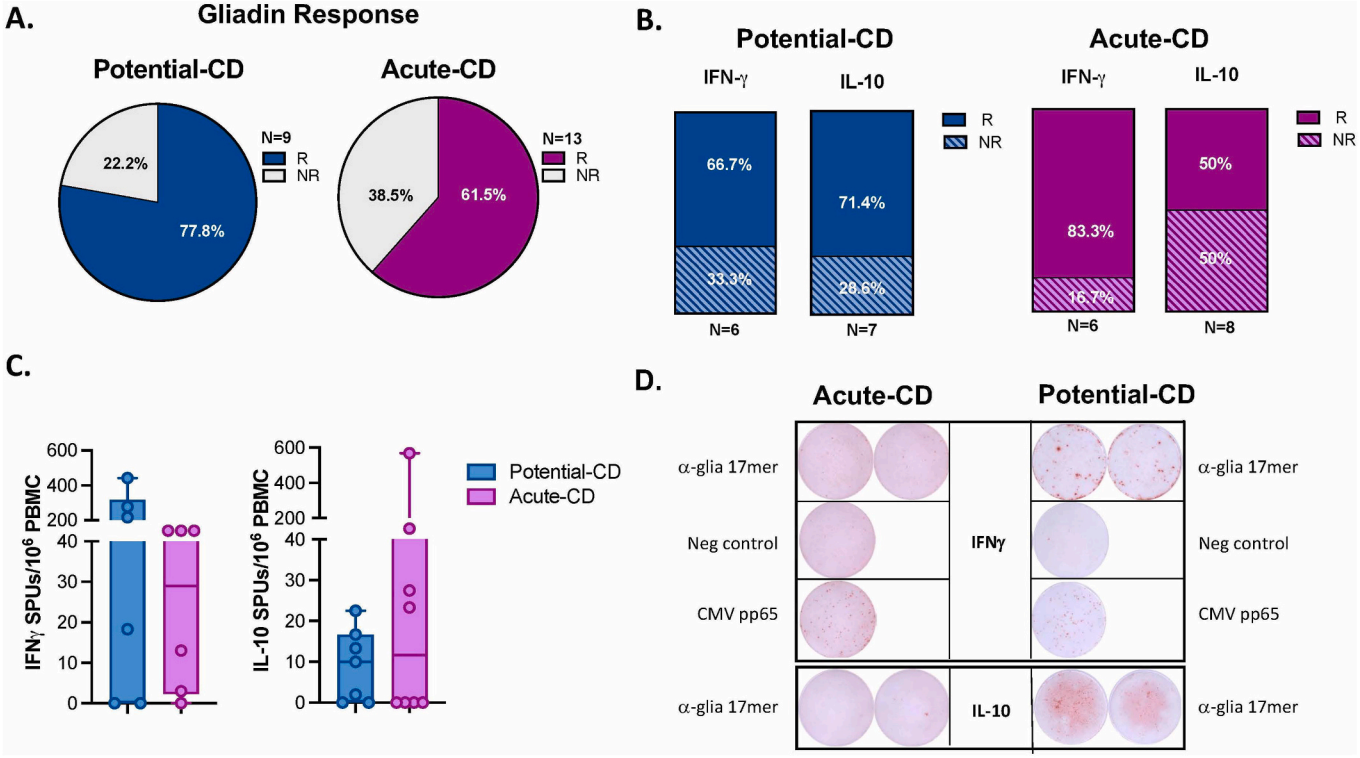

Fig. 2. Increased gliadin-specific IL-10 response in the peripheral blood of potential-CD patients. The response of PBMC to α-gliadin 17mer peptide was measured by IFN-γ and IL-10 ELISPOT assays. Stimulation with unrelated peptide served as negative control for specificity: patients with SFUs to α-glia 17mer > SFUs to control peptide were considered responders. CMV pp65-derived peptides and/or PHA served as positive controls of the assay. (A) Pie charts show the relative frequency of potential-CD and acute-CD patients with positive response to α-glia 17mer peptide, either by releasing IFN-γ, IL-10 or both. (B) Relative frequency of potential-CD (blue bars) and acute-CD (pink bars) patients responding to α-glia 17mer peptide by releasing IFN-γ and/or IL-10. (C) IFN-γ (left panel) and IL-10 (right panel) spot forming units (SFU)/10^6 PBMC from acute-CD and potential CD patients in response to α-glia 17mer peptide. (D) Representative wells from ELISPOT plates are reported. Control: unrelated scramble peptide, negative control; CMV pp65: PepTivator CMV pp65. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

alterations in the myeloid compartment (Fig. 6D and not shown).

In GFD-CD patients we observed a consistent reduction of IL-12p70 and IFN-γ in plasma compared to those detected in acute-CD patients (Fig. 7A), while no major differences were found in the proportion of circulating T cells (not shown) and in the peripheral T-cell response to gliadin (Fig. 7B, Table S7). Therefore, GFD regimen results in changes in leucocyte infiltrates, with increased density of regulatory cells and reduction of inflammation both in periphery and in the gut.

4. Discussion

Our study provides a comprehensive characterization of the immune compartments in PB and gut of potential-CD, acute-CD, and GFD-CD patients. A peripheral inflammatory signature characterizes CD patients, regardless of mucosal damage, while gluten deprivation restores steady state in the periphery. Circulating gliadin-specific effector T cells (IFN-γ^+) were mainly found in PB of acute-CD patients, whereas a prominent frequency of subjects with circulating gliadin-specific IL-10-secreting T cells was observed in the potential-CD group. The amount and composition of intestinal infiltrate well correlated with disease stage: the most abundant infiltrate, prevalently composed of lymphoid cells, was found in acute-CD patients, while increased frequency of IL-10-secreting DC-10 was found exclusively in biopsies from potential-CD subjects. Inhibition of IL-10 and TGF-β during gliadin stimulation, increased IFN-γ secretion by intestinal T-cell lines from CD patients, suggesting that differentiation of gliadin-specific Tregs occured during gluten exposure. On GFD, despite shrinkage of the overall small intestinal infiltrate, high frequency of TCRγδ$^+$ and Tr1 cells persisted. These results suggest that IL-10-related regulatory cells play a role in CD progression and/or remission.

As expected, in the periphery neither the lymphoid nor the myeloid compartments were affected during exposure to or deprivation of gluten. Conversely, a subset of CD patients displays an overall peripheral pro-inflammatory profile, mainly characterized by increase of Th1 cytokines in plasma. Inflammation is likely driven by gluten intake rather than degree of tissue damage, as it is detactable in acute-CD and potential-CD patients. In line with these results, it has recently reported that the amount of gluten intake during the second year of life correlates with an inflammatory profile in serum cytokines at 36 months of age and development of CD [31]. Interestingly, dysregulation of inflammatory cytokines in serum was observed long before CD onset and before gluten introduction in the diet (at 4 months of age) in those infants who developed CD later in life, suggesting that the natural history of CD is associated with systemic hallmarks of inflammation [31]. Furthermore, single-cell RNA sequencing of CD patients PBMC prior and after diagnosis showed that deregulation of CD4$^+$ T cells occurs before disease onset, with up-regulation of genes affecting T-cell proliferation [32]. Accordingly, we report that in potential-CD subjects inflammatory cytokines and peripheral T-cell activation/proliferation precede overt disease and intestinal tissue damage. Longer follow up studies are required to correlate serum cytokines and T-cell activation to outcome of potential-CD evolution in single patients.

In line with previous reports [33], we found an abundant inflammatory infiltrate in acute-CD, mainly composed of lymphoid cells. Despite their unclear role in CD pathogenesis, abundance of TCRγδ$^+$ T cells was reported in CD mucosa, with discrepant results in acute-CD *vs* potential-CD [20,34–37]. Our study did not reveal differences in TCRγδ$^+$ T cell frequency in acute-CD *vs* potential-CD. Of notice, our analyses

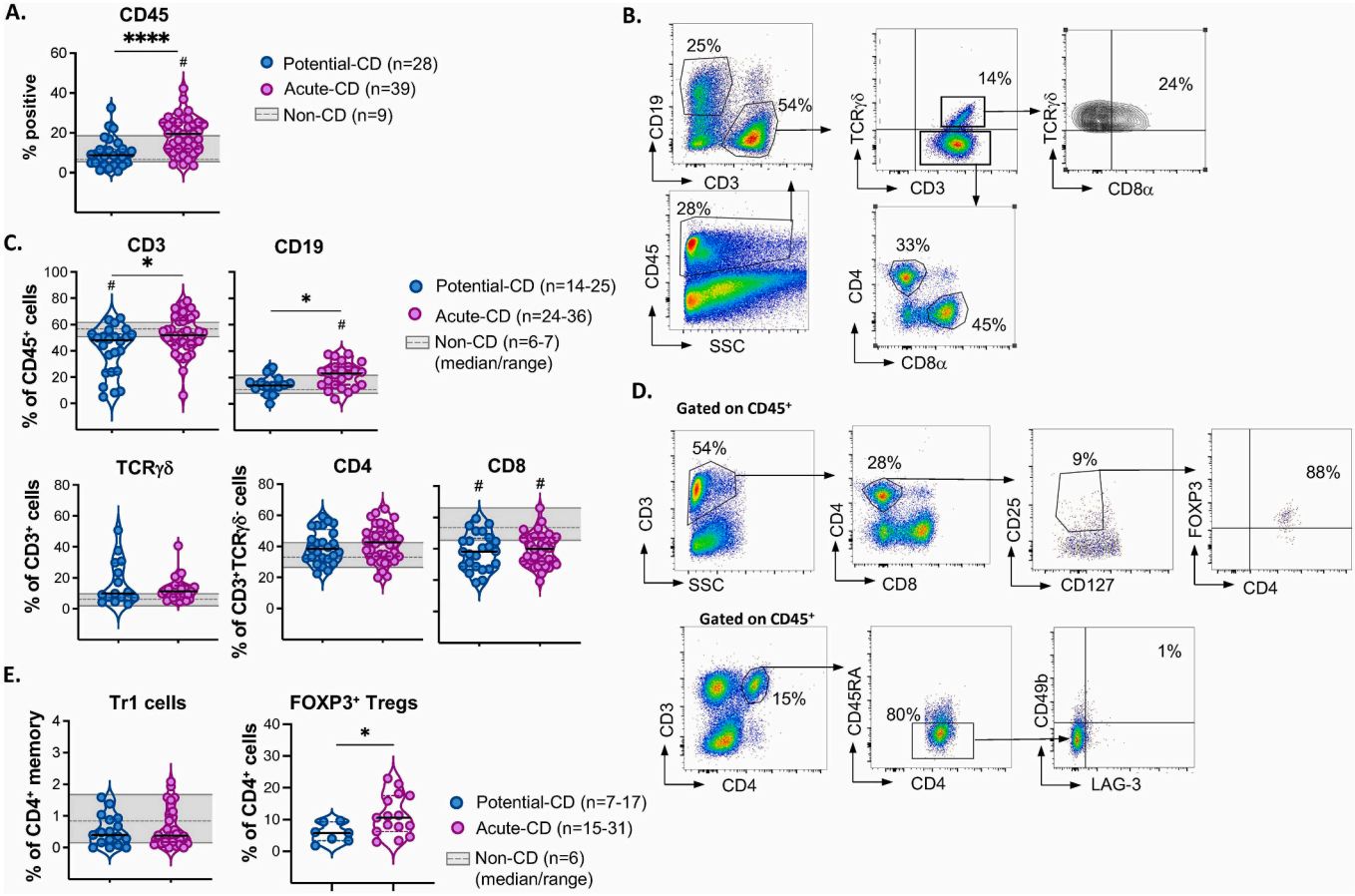

Fig. 3. Lymphoid cell infiltrate in duodenal biopsies of untreated CD patients. The proportion of the major leukocyte subsets was analyzed by multiflow cytometry of freshly isolated cells from gut biopsies of potential-CD and acute-CD patients and age-matched non-CD controls. (A) The percentage of $CD45^+$ cells is reported in the graph. (B) Representative gating strategy used to identify the frequencies of T ($CD3^+$, $CD4^+$, and $CD8^+$), B ($CD19^+$) and $CD3^+TCR\gamma\delta^+$. Numbers in the plots represent percentage of positive cells in the parental gate. Representative plots from an acute-CD patient are shown. (C) The percentage of $CD3^+$ and $CD19^+$ within $CD45^+$ cells, of $TCR\gamma\delta^+$, $CD4^+$ and $CD8^+$ within $CD3^+TCR\gamma\delta^-$ T cells, according to the gating strategy shown in (B) is reported. (D) Representative gating strategy used to identify the frequencies of regulatory T cells, either Tr1 ($CD4^+CD45RA^-CD49b^+LAG\text{-}3^+$), and $FOXP3^+$ Treg ($CD4^+CD127^-CD25^+FOXP3^+$) cells. (E) The percentage of Tr1 ($CD49b^+LAG\text{-}3^+$) within the memory $CD4^+$ T cell compartment and of $FOXP3^+$ Tregs ($CD127^-CD25^+FOXP3^+$) within $CD4^+$ T cell gate in the indicated numbers of potential-CD and acute-CD patients is reported. Range non-CD controls = 25th–75th percentile. Violin plots are shown, dots represent single patients. Kruskal-Wallis test with Benjamini-Hochberg FDR correction was used to define statistical significance. Mann Whitney *U* test was applied to two-group comparisons. #: Potential-CD or Acute-CD patients *vs* non-CD. *: Potential-CD *vs* Acute-CD patients. * or # ≤0.05; **** ≤0.0001.

were performed on cells derived from collagenase digestion of the entire gut mucosa (epithelium plus lamina propria), whilst other studies have specifically analyzed the epithelium compartment, which may account for discrepancies and may limit the understanding of the changes occurring in the distinct compartments. On the same line, the frequency of $CD8^+$ T cells, not exclusively intraepithelial, was found lower in CD patients than in non-CD samples. However, given the increased number of infiltrating $CD45^+$ cells in acute-CD patients, the overall number of cytotoxic T cells, which mediate the mucosal damage in CD [38], is expected to exceed that of a healthy mucosa. Furthermore, despite enriched frequency of $FOXP3^+$ Tregs in gut of acute-CD patients, the effector cell compartment lacks efficient control, likely due to dysfunction/plasticity of Tregs in the inflamed tissue [39].

While presence of IDO^+ tolDC in the intestinal mucosa of CD patients was previously reported [40,41], we identified for the first time DC-10, a subset of tolDC specialized in IL-10 secretion and induction of Tr1 cells [19,42], in gut biopsies from CD and non-CD subjects. Type 2 tTG (tTG2) is expressed on the cell surface of DC from intestinal celiac lesions and it can be actively endocytosed, leading to the hypothesis, not formerly demonstrated, that cell surface-associated tTG2 on APCs could be involved in gluten uptake, subsequent deamidation, and Ag-presentation [43]. Our recent transcriptome profiling of *in vitro* generated DC-10 demonstrated >10-fold higher expression of tTG2 gene compared to that detected in conventional DC [44]. This observation, together with our finding that DC-10 frequency is significantly higher in potential-CD than in acute-CD, led us to speculate that this cell subset may be involved in gliadin-derived peptides processing and presentation in a pro-tolerogenic manner to T cells within the potential-CD gut mucosa, thus orchestrating gluten tolerance maintenance.

Gliadin-specific effector ($IFN\gamma^+$) T cells are detectable in circulation regardless of degree of mucosal status. We also found a strong IFNγ response to gliadin by intestinal T-cell lines derived from potential-CD patients' biopsies, supporting that effector T cells are also present in the gut mucosa prior to tissue damage. IL-10 response was more frequently found in the potential-CD group than in patients with atrophic mucosa or on GFD. This observation may reflect presence of gliadin-specific Tr1 cells in the gut mucosa. Indeed, the combined IL-10/TGFβ signaling blockade boosted IFN-γ production by gliadin-specific intestinal T-cell lines from both potential- and acute-CD mucosa, suggesting that during *in vivo* exposure to gliadin Tr1 cells are induced. Furthermore, a higher proportion of DC-10 was exclusively detected in potential-CD patients. Thus it can be speculated that activation of gliadin-specific effector T cells within the gut mucosa is controlled by tolerogenic DC-10. In line with this hypothesis, *in vitro* activation of

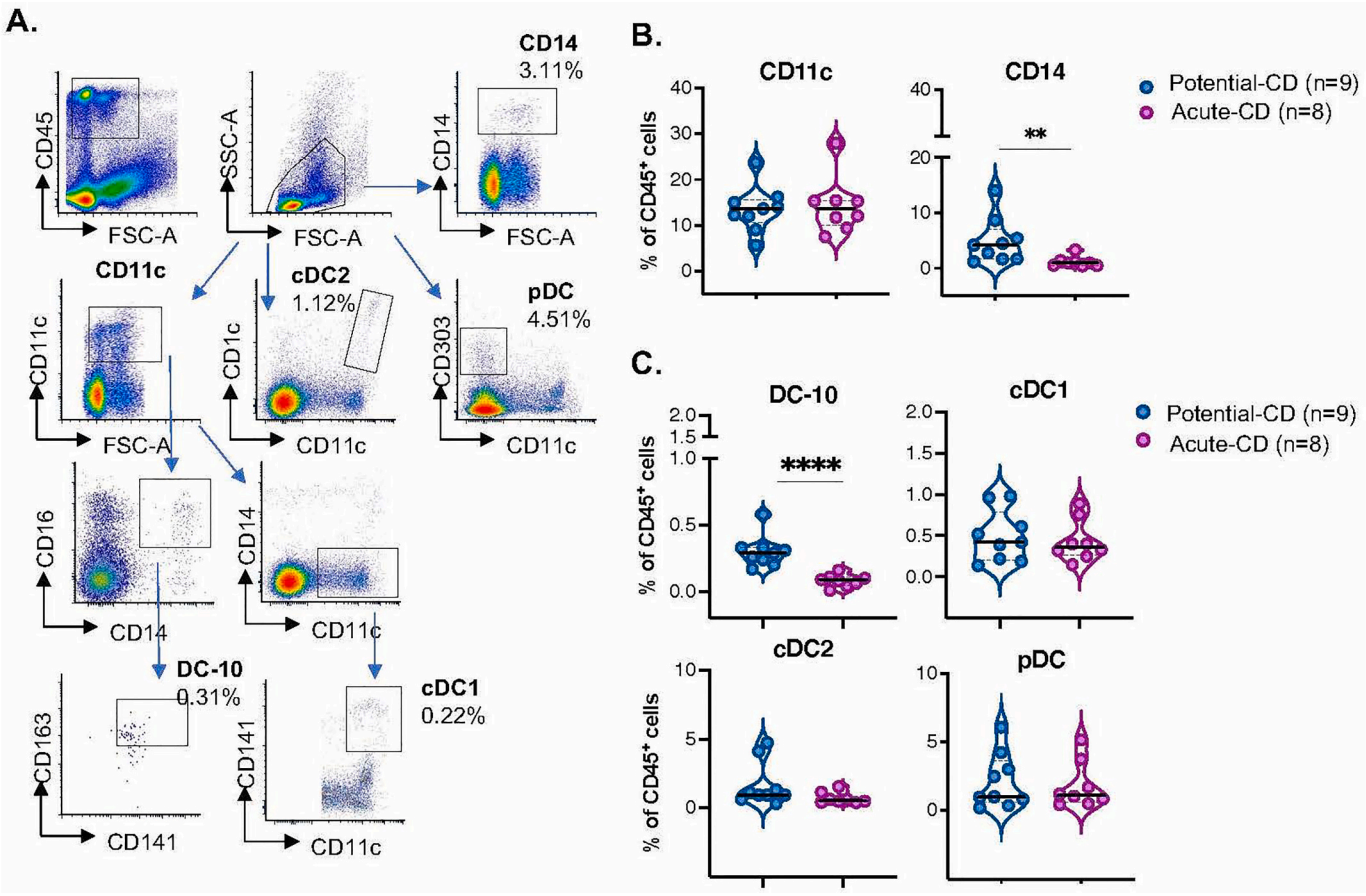

Fig. 4. Myeloid cell infiltrate in duodenal biopsies of untreated CD patients. Monocytes ($CD14^+$) and dendritic cell (DC) subsets were identified in cell suspensions freshly isolated from gut biopsies of potential-CD patients, CD patients with atrophic mucosa (acute-CD), and age-matched non-CD controls (non-CD), by multi-parametric flow cytometry. (A) Representative gating strategy used to identify the frequencies of monocytes ($CD14^+$), myeloid cells ($CD11c^+$), classical (c)DC2 ($CD11c^+CD1c^+$) and cDC1 ($CD14^-CD11c^+CD141^+$), plasmacytoid (p)DC ($CD11c^+CD303^+$), and DC-10 ($CD11c^+CD14^+CD16^+CD14^+CD163^+$) cells. Representative plots from a potential-CD patient is shown. Dot plots show viable intestinal mononuclear cells in the SSC-A/FSC-A gate, and the numbers in the plots indicate the percentage of positive cells for indicated surface cell markers. Percentage of (B) $CD11c^+$ and $CD14^+$ cells, (C) of DC-10, cDC1, cDC2, and pDC within the $CD45^+$ cells in the indicated number of potential-CD and acute-CD patients is plotted in the graphs. Violin plots are shown, each dot represents single patients. Mann-Whitney U test was used to define statistical significance; $^{**}p \leq 0.01$; $^{****}p \leq 0.0001$.

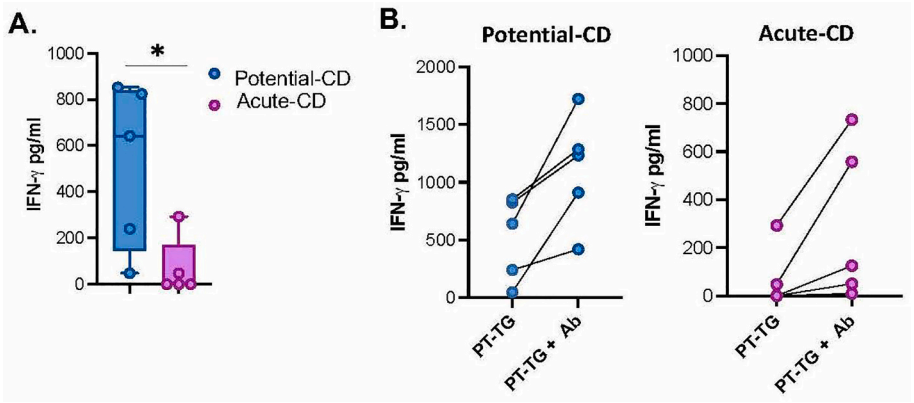

Fig. 5. IL-10 producing intestinal cells control gliadin-specific T-cell response in the gut of untreated CD patients. (A) IFN-γ production by gliadin-specific T-cell lines generated from jejunal biopsies of acute-CD ($n = 5$) and potential-CD (n = 5) patients. (B) IFN-γ production by gliadin-specific T-cell lines generated from jejunal biopsies of acute-CD (n = 5) and potential-CD (n = 5) patients in the absence (PT-TG) or presence of anti-IL-10R and anti-TGF-β (PT-TG+ Ab) mAbs. T-cell lines were stimulated for 48 h with irradiated autologous or HLA-matched immortalized B-cells (EBV) pulsed with deamidated gliadin digests (PT-TG). IFN-γ in culture supernatants was quantified by ELISA. Plots report IFN-γ values detected in cell culture supernatants from T-cell lines stimulated with EBV in presence of PT-TG *minus* IFN-γ values detected in T-cell culture supernatants stimulated with EBV only (internal control). Box and whiskers (min to max) are shown, each dot represents an independent donor-derived T cell line. Paired *t*-test, $^{*}p \leq 0.05$.

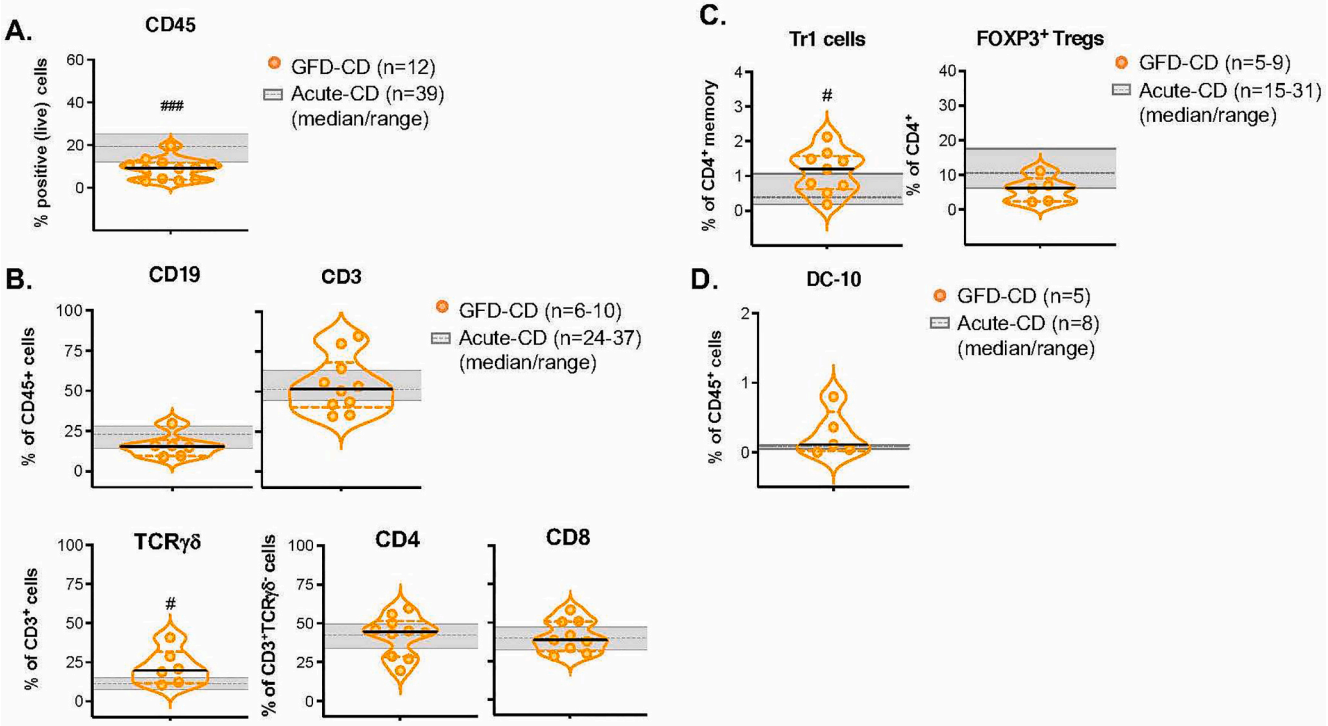

Fig. 6. Gluten free diet shapes composition of immune cells infiltrating the gut mucosa of CD patients. The proportion of the major leukocyte subsets was analyzed by flow cytometry in cell suspensions freshly isolated from gut biopsies of CD patients on gluten free diet (GFD-CD) and CD patients with atrophic gut mucosa (acute-CD), according to the gating strategy shown in Fig. 3. The percentage (A) of $CD45^+$ cells, (B) of $CD19^+$ and $CD3^+$ within $CD45^+$ cells, of $TCR\gamma\delta^+$ within $CD3^+$ T cells, $CD4^+$ and $CD8^+$ within $CD3^+TCR\gamma\delta^-$ T cells, (C) of Tr1 ($CD49b^+LAG\text{-}3^+$) within the memory $CD4^+$ T cell compartment, of $FOXP3^+$ Treg ($CD4^+CD127^-CD25^+FOXP3^+$) within $CD4^+$ T cells, (D) and of DC-10 ($CD11c^+CD14^+CD16^+CD14^+CD163^+$) cells within the $CD45^+$ cells in the indicated numbers of GFD-CD patients is shown. Range Acute-CD = 25th–75th percentile. Violin pots are shown, dots represent single patients. Mann Whitney U test was used to define statistical significance; #: GFD-CD *vs* acute-CD patients. # $p \leq 0.05$; ### $p \leq 0.001$.

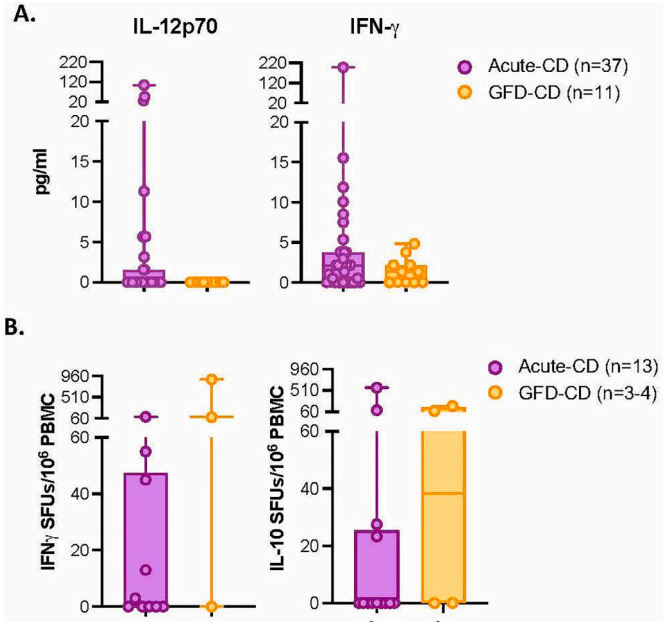

Fig. 7. Gluten free diet modulates the inflammatory state of untreated CD patients. A. The concentration of IL-12p70 and IFN-γ in the plasma of celiac patients with intestinalatrophy (acute-CD; $n = 37$) and on gluten free diet (GFD-CD, $n = 11$) was evaluated using a multi-bead array. Box and whiskers (minimum to maximum) are presented, dots represent individual patients.

gliadin-specific intestinal T-cell lines from potential-CD patients, in the absence of DC-10, leads to T-cell expansion and IFN-γ release. On the other hand, the low proportion of DC-10 in the gut of acute-CD subjects cannot control gliadin-specific effector T cell activation, resulting in mucosal inflammation, release of inflammatory mediators, and consequent cytotoxic cell-mediated tissue damage. These results are in line with previous findings showing that IL-10-RNA expression was upregulated in biopsies from potential-CD patients with intact mucosa *vs* those progressing to Marsh 1 [14]. Therefore, the low grade of inflammation in potential-CD patients could likely be due to active IL-10-dependent regulatory mechanisms preventing the progression toward mucosal damage.

While in GFD-CD patients we did not detect significant variations in circulating leucocytes, we found that the pro-inflammatory cytokines detected in acute-CD patients disappeared. Moreover, upon GFD the overall frequency of inflammatory cells in the gut is reduced, while $TCR\gamma\delta^+$ T cells persist and the relative frequency of Tr1 cells increased, likely due to shrinkage of the effector T-cell compartment, after gluten retrieval from diet, in accordance with previous reports [18,30,37]. These changes are in line with the idea that prolonged chronic inflammation permanently reshapes the composition of tissue-infiltrating immune cells [45], regardless of mucosal healing. Despite Tr1 cell persistence, exposure of GFD-CD patients to gluten rapidly induces gluten-specific $CD4^+$ T cells reactivation and consequent gastrointestinal symptoms [46,47], indicating that deprivation of gluten does not *per se* establish a self-sustaining tolerogenic circuit that protects tissues from damage, as occurring in potential-CD patients.

5. Conclusions

Overall, our study strongly supports a fundamental role for IL-10 in

suppressing pathological T-cell responses to gliadin. We newly identified DC-10 as peculiar mark of potential-CD, and we hypothetize that they can contribute to maintain mucosal health by controlling gliadin-specific T-cell response, at a disease stage in which peripheral inflammatory skewing and differentiation of gliadin-specific effector T cells are already in place. The mechanisms underlying the progression toward the acute-CD stage in untreated patients remain elusive. Although we cannot exclude that other regulatory mechanisms, such as IL-4 producing T cells [26], may be involved in contrasting the inflammatory cascade in potential-CD to acute-CD stage transition, *in situ* differentiation/recruitement of IL-10-producing tolerogenic myeloid cells is fundamental for mucosal homeostasis maintenance.

Funding

This work was supported by the Italian Telethon Foundation (TeleG1 2016–2021 to SG); the Italian Ministry of Health (RF-2016-02361372 to SG); the Vita-Salute San Raffaele University (Fellowship to VB).

Data availability

Datasets included in the present Manuscript are publicly available at the San Raffaele Open Research Data Repository under the link DOI:10.17632/nhzr85fts6.1.

Acknowledgements

We thank B. Mazzi, Immuno-hematology and transfusion medicine Unit of OSR, Milan, for help in setting up of HLA-DQ8/DQ2 screening. Thanks to patients and their families for their participation in our studies.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clim.2024.109923.

References

[1] S. Husby, S. Koletzko, I.R. Korponay-Szabo, M.L. Mearin, A. Phillips, R. Shamir, R. Troncone, K. Giersiepen, D. Branski, C. Catassi, M. Lelgeman, M. Maki, C. Ribes-Koninckx, A. Ventura, K.P. Zimmer, E.W.G.o.C.D. Diagnosis, E.G. Committee, H. European Society for Pediatric Gastroenterology, Nutrition, European Society for Pediatric Gastroenterology, hepatology, and nutrition guidelines for the diagnosis of coeliac disease, J. Pediatr. Gastroenterol. Nutr. 54 (2012) 136–160.

[2] K.A. Scherf, C. Catassi, F.G. Chirdo, P.J. Ciclitira, C.F. Feighery, C. Gianfrani, F. Koning, K.E.A. Lundin, S. Masci, D. Schuppan, M.J.M. Smulders, O. Tranquet, R. Troncone, P. Koehler, Statement of the Prolamin working group on the determination of gluten in fermented foods containing partially hydrolyzed gluten, Front. Nutr. 7 (2020) 626712.

[3] G. Caio, R. Ciccocioppo, G. Zoli, R. De Giorgio, U. Volta, Therapeutic options for coeliac disease: what else beyond gluten-free diet? Dig. Liver Dis. 52 (2020) 130–137.

[4] R. Iversen, L.M. Sollid, The Immunobiology and pathogenesis of celiac disease, Annu. Rev. Pathol. 18 (2023) 47–70.

[5] F. Paparo, E. Petrone, A. Tosco, M. Maglio, M. Borrelli, V.M. Salvati, E. Miele, L. Greco, S. Auricchio, R. Troncone, Clinical, HLA, and small bowel immunohistochemical features of children with positive serum antiendomysium antibodies and architecturally normal small intestinal mucosa, Am. J. Gastroenterol. 100 (2005) 2294–2298.

[6] R. Auricchio, A. Tosco, E. Piccolo, M. Galatola, V. Izzo, M. Maglio, F. Paparo, R. Troncone, L. Greco, Potential celiac children: 9-year follow-up on a gluten-containing diet, Am. J. Gastroenterol. 109 (2014) 913–921.

[7] M.J. Girard-Madoux, J.L. Ober-Blobaum, L.M. Costes, J.M. Kel, D.J. Lindenbergh-Kortleve, I. Brouwers-Haspels, A.P. Heikema, J.N. Samsom, B.E. Clausen, IL-10 control of CD11c+ myeloid cells is essential to maintain immune homeostasis in the small and large intestine, Oncotarget 7 (2016) 32015–32030.

[8] L.M.M. Costes, D.J. Lindenbergh-Kortleve, L.A. van Berkel, S. Veenbergen, H.R. C. Raatgeep, Y. Simons-Oosterhuis, D.H. van Haaften, J.J. Karrich, J.C. Escher, M. Groeneweg, B.E. Clausen, T. Cupedo, J.N. Samsom, IL-10 signaling prevents gluten-dependent intraepithelial CD4(+) cytotoxic T lymphocyte infiltration and epithelial damage in the small intestine, Mucosal Immunol. 12 (2019) 479–490.

[9] M.F. Du Pre, A.E. Kozijn, L.A. van Berkel, M.N. ter Borg, D. Lindenbergh-Kortleve, L.T. Jensen, Y. Kooy-Winkelaar, F. Koning, L. Boon, E.E. Nieuwenhuis, L.M. Sollid, L. Fugger, J.N. Samsom, Tolerance to ingested deamidated gliadin in mice is maintained by splenic, type 1 regulatory T cells, Gastroenterology 141 (2011) 610–620, 620 e611–612.

[10] T.L. Freitag, J.R. Podojil, R.M. Pearson, F.J. Fokta, C. Sahl, M. Messing, L. C. Andersson, K. Leskinen, P. Saavalainen, L.I. Hoover, K. Huang, D. Phippard, S. Maleki, N.J.C. King, L.D. Shea, S.D. Miller, S.K. Meri, D.R. Getts, Gliadin nanoparticles induce immune tolerance to gliadin in mouse models of celiac disease, Gastroenterology 158 (2020) 1667–1681 e1612.

[11] M.S. Lopez, M.M. Tiscornia, M.B. Dicarlos, P.D. Zapata, IL-10 gene promoter region polymorphisms and their association with celiac disease, Rev. Esp. Enferm. Dig. 112 (2020) 915–920.

[12] M. Aflatoonian, G. Sivandzadeh, M. Morovati-Sharifabad, S.R. Mirjalili, M. J. Akbarian-Bafghi, H. Neamatzadeh, Associations of Il-6 -174g>C and Il-10 -1082a>G polymorphisms with susceptibility to celiac disease: evidence from a Meta-analysis and literature review, Arq. Gastroenterol. 56 (2019) 323–328.

[13] D. Barisani, S. Ceroni, R. Meneveri, B.M. Cesana, M.T. Bardella, IL-10 polymorphisms are associated with early-onset celiac disease and severe mucosal damage in patients of Caucasian origin, Genet. Med. 8 (2006) 169–174.

[14] M. Borrelli, V.M. Salvati, M. Maglio, D. Zanzi, K. Ferrara, S. Santagata, D. Ponticelli, R. Aitoro, G. Mazzarella, G. Lania, C. Gianfrani, R. Auricchio, R. Troncone, Immunoregulatory pathways are active in the small intestinal mucosa of patients with potential celiac disease, Am. J. Gastroenterol. 108 (2013) 1775–1784.

[15] V.M. Salvati, G. Mazzarella, C. Gianfrani, M.K. Levings, R. Stefanile, B. De Giulio, G. Iaquinto, N. Giardullo, S. Auricchio, M.G. Roncarolo, R. Troncone, Recombinant human interleukin 10 suppresses gliadin dependent T cell activation in ex vivo cultured coeliac intestinal mucosa, Gut 54 (2005) 46–53.

[16] C.R. Meijer, R. Auricchio, H. Putter, G. Castillejo, P. Crespo, J. Gyimesi, C. Hartman, S. Kolacek, S. Koletzko, I. Korponay-Szabo, E.M. Ojinaga, I. Polanco, C. Ribes-Koninckx, R. Shamir, H. Szajewska, R. Troncone, V. Villanacci, K. Werkstetter, M.L. Mearin, Prediction models for celiac disease development in children from high-risk families: data from the PreventCD cohort, Gastroenterology 163 (2022) 426–436.

[17] A. Camarca, R. Auricchio, S. Picascia, O. Fierro, M. Maglio, E. Miele, B. Malamisura, L. Greco, R. Troncone, C. Gianfrani, Gliadin-reactive T cells in Italian children from preventCD cohort at high risk of celiac disease, Pediatr. Allergy Immunol. 28 (2017) 362–369.

[18] C. Gianfrani, M.K. Levings, C. Sartirana, G. Mazzarella, G. Barba, D. Zanzi, A. Camarca, G. Iaquinto, N. Giardullo, S. Auricchio, R. Troncone, M.G. Roncarolo, Gliadin-specific type 1 regulatory T cells from the intestinal mucosa of treated celiac patients inhibit pathogenic T cells, J. Immunol. 177 (2006) 4178–4186.

[19] S. Gregori, D. Tomasoni, V. Pacciani, M. Scirpoli, M. Battaglia, C.F. Magnani, E. Hauben, M.G. Roncarolo, Differentiation of type 1 T regulatory cells (Tr1) by tolerogenic DC-10 requires the IL-10-dependent ILT4/HLA-G pathway, Blood 116 (2010) 935–944.

[20] S. Vitale, M. Maglio, S. Picascia, I. Mottola, E. Miele, R. Troncone, R. Auricchio, C. Gianfrani, Intestinal cellular biomarkers of mucosal lesion progression in pediatric celiac disease, Pharmaceutics 13 (2021).

[21] C. Gianfrani, R.A. Siciliano, A.M. Facchiano, A. Camarca, M.F. Mazzeo, S. Costantini, V.M. Salvati, F. Maurano, G. Mazzarella, G. Iaquinto, P. Bergamo, M. Rossi, Transamidation of wheat flour inhibits the response to gliadin of intestinal T cells in celiac disease, Gastroenterology 133 (2007) 780–789.

[22] G. Amodio, A. Mandelli, R. Curto, P.M.V. Rancoita, A. Stabilini, R. Bonfanti, M. de Pellegrin, E. Bosi, C. Di Serio, M. Battaglia, S. Gregori, Altered frequency and phenotype of HLA-G-expressing DC-10 in type 1 diabetes patients at onset and in subjects at risk to develop the disease, Front. Immunol. 12 (2021) 750162.

[23] N. Gagliani, C.F. Magnani, S. Huber, M.E. Gianolini, M. Pala, P. Licona-Limon, B. Guo, D.R. Herbert, A. Bulfone, F. Trentini, C. Di Serio, R. Bacchetta, M. Andreani, L. Brockmann, S. Gregori, R.A. Flavell, M.G. Roncarolo, Coexpression of CD49b and LAG-3 identifies human and mouse T regulatory type 1 cells, Nat. Med. 19 (2013) 739–746.

[24] A. Kurki, E. Kemppainen, P. Laurikka, K. Kaukinen, K. Lindfors, The use of peripheral blood mononuclear cells in celiac disease diagnosis and treatment, expert rev, Gastroenterol. Hepatol. 15 (2021) 305–316.

[25] V. Abadie, V. Discepolo, B. Jabri, Intraepithelial lymphocytes in celiac disease immunopathology, Semin. Immunopathol. 34 (2012) 551–566.

[26] S. Vitale, V. Santarlasci, A. Camarca, S. Picascia, A.D. Pasquale, M. Maglio, E. Maggi, L. Cosmi, F. Annunziato, R. Troncone, R. Auricchio, C. Gianfrani, The intestinal expansion of TCRgammadelta(+) and disappearance of IL4(+) T cells suggest their involvement in the evolution from potential to overt celiac disease, Eur. J. Immunol. 49 (2019) 2222–2234.

[27] M. Raki, A.C. Beitnes, K.E. Lundin, J. Jahnsen, F.L. Jahnsen, L.M. Sollid, Plasmacytoid dendritic cells are scarcely represented in the human gut mucosa and are not recruited to the celiac lesion, Mucosal Immunol. 6 (2013) 985–992.

[28] A. Camarca, R.P. Anderson, G. Mamone, O. Fierro, A. Facchiano, S. Costantini, D. Zanzi, J. Sidney, S. Auricchio, A. Sette, R. Troncone, C. Gianfrani, Intestinal T cell responses to gluten peptides are largely heterogeneous: implications for a peptide-based therapy in celiac disease, J. Immunol. 182 (2009) 4158–4166.

[29] J. Ritter, K. Zimmermann, K. Johrens, S. Mende, A. Seegebarth, B. Siegmund, S. Hennig, K. Todorova, A. Rosenwald, S. Daum, M. Hummel, M. Schumann, T-cell repertoires in refractory coeliac disease, Gut 67 (2018) 644–653.

[30] M.R. Dunne, L. Elliott, S. Hussey, N. Mahmud, J. Kelly, D.G. Doherty, C.F. Feighery, Persistent changes in circulating and intestinal gammadelta T cell subsets, invariant natural killer T cells and mucosal-associated invariant T cells in children and adults with coeliac disease, PLoS One 8 (2013) e76008.

[31] R. Auricchio, I. Calabrese, M. Galatola, D. Cielo, F. Carbone, M. Mancuso, G. Matarese, R. Troncone, S. Auricchio, L. Greco, Gluten consumption and inflammation affect the development of celiac disease in at-risk children, Sci. Rep. 12 (2022) 5396.
[32] A.D. Ramirez-Sanchez, X. Chu, R. Modderman, Y. Kooy-Winkelaar, S. Koletzko, I. R. Korponay-Szabo, R. Troncone, C. Wijmenga, L. Mearin, S. Withoff, I.H. Jonkers, Y. Li, Single-cell RNA sequencing of peripheral blood mononuclear cells from pediatric coeliac disease patients suggests potential Pre-seroconversion markers, Front. Immunol. 13 (2022) 843086.
[33] J. Valle, J.M.T. Morgado, J. Ruiz-Martin, A. Guardiola, M. Lopes-Nogueras, A. Garcia-Vela, B. Martin-Sacristan, L. Sanchez-Munoz, Flow cytometry of duodenal intraepithelial lymphocytes improves diagnosis of celiac disease in difficult cases, United European Gastroenterol J 5 (2017) 819–826.
[34] K. Basu, H. Creasey, N. Bruggemann, J. Stevens, D. Bloxham, J.M. Woodward, Diagnosis of coeliac disease by flow cytometry of intraepithelial lymphocytes: a new 'gold' standard? Front. Gastroenterol. 13 (2022) 119–125.
[35] B. Burgueno Gomez, C. Escudero-Hernandez, R. de Pedro, E. Montalvillo, D. Bernardo, E. Garcia-Lagarto, J.A. Garrote Adrados, E. Arranz Sanz, L. Fernandez Salazar, Duodenal lymphogram as a complementary tool in the diagnosis of celiac disease in adults, Rev. Esp. Enferm. Dig. 112 (2020) 434–439.
[36] P. Nijeboer, T. van Gils, M. Reijm, R. Ooijevaar, B.I. Lissenberg-Witte, H.J. Bontkes, C.J.J. Mulder, G. Bouma, Gamma-Delta T lymphocytes in the diagnostic approach of coeliac disease, J. Clin. Gastroenterol. 53 (2019) e208–e213.
[37] G. Roy, F. Fernandez-Banares, M. Corzo, S. Gomez-Aguililla, C. Garcia-Hoz, C. Nunez, Intestinal and blood lymphograms as new diagnostic tests for celiac disease, Front. Immunol. 13 (2022) 1081955.
[38] B. Meresse, Z. Chen, C. Ciszewski, M. Tretiakova, G. Bhagat, T.N. Krausz, D. H. Raulet, L.L. Lanier, V. Groh, T. Spies, E.C. Ebert, P.H. Green, B. Jabri, Coordinated induction by IL15 of a TCR-independent NKG2D signaling pathway converts CTL into lymphokine-activated killer cells in celiac disease, Immunity 21 (2004) 357–366.
[39] D. Zanzi, R. Stefanile, S. Santagata, L. Iaffaldano, G. Iaquinto, N. Giardullo, G. Lania, I. Vigliano, A.R. Vera, K. Ferrara, S. Auricchio, R. Troncone, G. Mazzarella, IL-15 interferes with suppressive activity of intestinal regulatory T cells expanded in celiac disease, Am. J. Gastroenterol. 106 (2011) 1308–1317.
[40] F. Kheiri, M. Rostami-Nejad, D. Amani, A. Sadeghi, A. Moradi, E. Aghamohammadi, A. Sahebkar, M.R. Zali, Expression of tolerogenic dendritic cells in the small intestinal tissue of patients with celiac disease, Heliyon 8 (2022) e12273.
[41] T. Vorobjova, O. Uibo, K. Heilman, R. Uibo, Increased density of tolerogenic dendritic cells in the small bowel mucosa of celiac patients, World J. Gastroenterol. 21 (2015) 439–452.
[42] P.P. Chen, A.M. Cepika, R. Agarwal-Hashmi, G. Saini, M.J. Uyeda, D.M. Louis, B. Cieniewicz, M. Narula, L.C. Amaya Hernandez, N. Harre, L. Xu, B.C. Thomas, X. Ji, P. Shiraz, K.M. Tate, D. Margittai, N. Bhatia, E. Meyer, A. Bertaina, M. M. Davis, R. Bacchetta, M.G. Roncarolo, Alloantigen-specific type 1 regulatory T cells suppress through CTLA-4 and PD-1 pathways and persist long-term in patients, Sci. Transl. Med. 13 (2021) eabf5264.
[43] M. Raki, K.W. Schjetne, J. Stamnaes, O. Molberg, F.L. Jahnsen, T.B. Issekutz, B. Bogen, L.M. Sollid, Surface expression of transglutaminase 2 by dendritic cells and its potential role for uptake and presentation of gluten peptides to T cells, Scand. J. Immunol. 65 (2007) 213–220.
[44] D. Avancini, A. Testori, L. Fresolone, G. Andolfi, M. Vuono, V. Martinelli, F. R. Santoni de Sio, S. Gregori, Aryl hydrocarbon receptor activity downstream of IL-10 signaling is required to promote regulatory functions in human dendritic cells, Cell Rep. 42 (2023) 112193.
[45] A. Halper-Stromberg, B. Jabri, Maladaptive consequences of inflammatory events shape individual immune identity, Nat. Immunol. 23 (2022) 1675–1686.
[46] A. Camarca, A. Del Mastro, C. Gianfrani, Repertoire of gluten peptides active in celiac disease patients: perspectives for translational therapeutic applications, Endocr Metab Immune Disord Drug Targets 12 (2012) 207–219.
[47] G. Goel, A.J.M. Daveson, C.E. Hooi, J.A. Tye-Din, S. Wang, E. Szymczak, L. J. Williams, J.L. Dzuris, K.M. Neff, K.E. Truitt, R.P. Anderson, Serum cytokines elevated during gluten-mediated cytokine release in coeliac disease, Clin. Exp. Immunol. 199 (2020) 68–78.