World Journal of Gastroenterology

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World J Gastroenterol 2025 March 28; 31(12): 97120

DOI: 10.3748/wjg.v31.i12.97120

ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Case Control Study

Decreased gene expression of interleukin 2 receptor subunit γ (CD132) in tissues of patients with Crohn's disease

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Specialty type: Gastroenterology and hepatology

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade B, Grade B, Grade C, Grade D

Novelty: Grade A, Grade B, Grade B, Grade C

Creativity or Innovation: Grade A, Grade B, Grade B, Grade C

Scientific Significance: Grade A, Grade B, Grade B, Grade C

P-Reviewer: Ahmad F; Alshimerry AF; Zhou C

Received: May 23, 2024

Revised: September 20, 2024

Accepted: October 25, 2024

Published online: March 28, 2025

Processing time: 306 Days and 22.1 Hours

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Abstract

BACKGROUND

A deficiency of γδ T cells has been described in Crohn's disease (CD).

AIM
To analyze the gene expression of interleukin 7 (IL-7) and its receptors in the tissues of patients with CD.

METHODS
We studied the peripheral blood of 80 patients with CD, comparing them with a group of 80 healthy subjects. The number and apoptosis of αβ and γδ T cells in peripheral blood and the proportion of αβ and γδ T cells in the intestinal tissues of patients with CD (n = 25) were studied. The gene and protein expression of IL-7, IL-2 receptor subunit γ [cluster of differentiation 132 (CD132)], receptor α (CD127), and caspase-3 in tissues was analyzed by quantitative PCR. Serum IL-7 levels were also analyzed.

RESULTS
In patients with CD, a decreased number of γδ T cells and an increase in the apoptosis of CD56+ αβ and γδ T cells in peripheral blood was observed (P < 0.0001 and P < 0.01) respectively, and there was an inverse correlation among T subsets and their apoptosis. In addition, IL-7 gene expression and IL-7 protein in the tissues of these patients were increased. The titers of caspase-3 in tissues were low *vs* control group (P > 0.01). The percentage of CD8+γδ T cells decreased in tissues (P < 0.01), and was directly related to IL-7 levels in peripheral blood. The expression of IL-2 receptor subunit γ (CD132) was greatly decreased in the tissues of patients with CD (P < 0.05).

CONCLUSION
There may be a cause-effect relationship between the lower gene expression of the IL-2 receptor subunit γ (CD132) in tissues of patients with CD and γδ T cells immunodeficiency.

Key Words: Crohn's disease; Interleukin 7; Interleukin 2 receptor subunit γ (CD 132); Caspase-3; γδ T cells

Core Tip: A deficiency in T cells, and specifically in γδ T cells, has been described in Crohn's disease (CD). Interleukin 7 (IL-7) is essential for the activation and proliferation of this lymphocyte's subsets. This work demonstrates a decrease in IL-2 receptor subunit γ [cluster of differentiation 132 (CD132)] in tissues of patients with CD. This could be related to the deficiency of γδ T cells, and could open new works and lines of research for the application of new therapies against this disease.

Citation: Andreu-Ballester JC, Hurtado-Marcos C, García-Ballesteros C, Pérez-Griera J, Izquierdo F, Ollero D, Jiménez A, Gil-Borrás R, Llombart-Cussac A, López-Chuliá F, Cuéllar C. Decreased gene expression of interleukin 2 receptor subunit γ (CD132) in tissues of patients with Crohn's disease. *World J Gastroenterol* 2025; 31(12): 97120
URL: https://www.wjgnet.com/1007-9327/full/v31/i12/97120.htm
DOI: https://dx.doi.org/10.3748/wjg.v31.i12.97120

INTRODUCTION

The exact cause of Crohn's disease (CD) remains unknown due to a lack of knowledge about its etiology. Recent studies suggest that defects affecting innate immune response after disruption of the intestinal epithelial barrier is the primary cause in CD[1,2]. In the immune system, γδ T cells, due to their frequent intraepithelial location in the mucous membranes and their functional properties, have qualities of innate and adaptive immunity, and constitute a first line of defense against attacks from the outside world[3]. We previously described a reduction in γδ T cell subsets in peripheral blood from patients with CD[4]. In addition, this reduction was repeated in all three clinical scenarios studied: New patients, active disease, and remission. Therefore, the study of γδ T cells is a valuable tool to understand the pathophysiology of CD[5].

Interleukin 7 (IL-7) plays an important role in the normal functioning of the immune system, exerting a signal necessary for the proliferation, differentiation, and survival of B, T, natural killer (NK), and other immune cells. It exerts its biological actions by binding to its specific receptor (IL-7R), a heterodimer consisting of two different subunits, IL-7Rα [cluster of differentiation 127 (CD127)] and IL-2 receptor subunit γ (CD132)[6]. Alterations in both IL-7 and its receptors are associated with immunodeficiency and other autoimmune and inflammatory diseases, including cancer. Therefore, IL-7 was proposed as a promising therapeutic approach for many diseases[7]. IL-7 signaling is required for all γδ T cell development. In fact, IL-7R-deficient mice develop few αβ T cells and B cells, but γδ T cells are absent, demonstrating that IL-7R signaling is critical for the development of thymic and extrathymic γδ T cells[8]. IL-7 is produced by the mesenchymal stromal cells of bone marrow and epithelial cells of the thymus, lymph nodes, skin, liver, and intestines. Thus, IL-7 expression by enterocytes is sufficient for the extrathymic development of γδ T cells[9,10]. We observed that levels of IL-7 in peripheral blood of patients with CD were decreased[11]. The role of IL-7 and its receptors in tissues of patients with CD was studied by Belarif *et al*[12]. In this paper, the authors described that the failure of inflammatory

bowel disease (IBD) treatments (CD and ulcerative colitis) is strongly associated with the overexpressed IL-7R signaling pathway of these patients, although only IL-7Rα (CD127) was analyzed[12].

Apoptosis or programmed cell death of T cells is an important mechanism to maintain peripheral immune tolerance. Observations by several laboratories showed that mucosal T cells of patients with IBD were highly resistant to apoptosis [13-18]. Due to these findings, it has been reasoned that the persistent T cell activation in the mucosa would explain that the inflammatory reaction in patients with IBD is resilient to resolution. However, apoptosis in peripheral blood lymphocytes and tissues of patients with CD was hardly studied. Similarly, there are no known studies on IL-7 in tissues and its relationship with apoptosis.

Our objective was to analyze the gene expression of IL-7 and its receptors in tissues of patients with CD, to determine the relationship between the concentration of this cytokine and the deficiency of γδ T cells.

MATERIALS AND METHODS

Study population

In this prospective case-control study, we studied the peripheral blood of 160 subjects, 80 patients with CD, comparing them with 80 healthy subjects (control group). In addition, we analyzed the tissues of 25 of these patients compared to a group of 25 healthy subjects. The tissues (intestinal biopsies) were obtained from patients with CD who underwent endoscopy and surgery at the Arnau de Vilanova Hospital in Valencia, Spain. The Lennard-Jones criteria were used for the diagnosis of CD[19]. Activity was measured using the CD activity index (CDAI)[20]. Patients with CD were divided according to three clinical scenarios: "New patients" with active CD presenting at diagnosis with no prior CD treatment, or with only treatment within the previous 24 hours, "remission" (CDAI < 150 for at least 12 months), and "active disease" (CDAI > 150 and signs and symptoms of disease). Control healthy subjects were required to have the following characteristics: They were not suffering from immunodeficiency or autoimmune or inflammatory diseases, had not been vaccinated in the last 3 months, and were not undergoing immunosuppressive therapy. Tissues from healthy subjects were biopsied by routine colonoscopies program to detect colon cancer and reported as normal. Cases and controls were matched for sex and age ± 5 years. The study was approved by the Research Ethics Committee of the Arnau de Vilanova-Lliria University Hospital.

Tissue sampling of intestinal biopsies

Three to five biopsies of ileum and colon were collected at 1-2 cm intervals from each subject. Some samples were fixed through immersion in a solution of 10% buffered formalin. Specimens were routinely processed by paraffin embedding. Tissue was stained by a hematoxylin-eosin staining method. Some samples were frozen without fixing for cellular and molecular analyses. Small biopsy samples of tissues were homogenized and washed in separation medium [phosphate-buffered saline (PBS), 1% fetal bovine serum (FBS), 1 mmol/L DTT, 1 mmol/L EDTA] for 15 minutes at 37 °C.

After centrifugation, the pellet was incubated in digestion medium consisting of 5% FBS, 0.5 mg/mL collagenase type VIII (Sigma-Aldrich, St. Louis, MO, United States) for 30 minutes at 37 °C by gentle shaking. Cells were passed through a mesh, centrifuged, and stained with lineage markers. Human lymphocytes were isolated from the intestinal mucosa of healthy individuals and patients with CD.

Cell isolation for analysis of γδ and αβ T cells and apoptosis evaluation

Blood cell counts were obtained using a cell counter (LH750; Beckman Coulter, Inc., Fullerton, CA, United States). An enrichment of the sample in mononuclear cells was obtained by density gradient centrifugation from EDTA anticoagulated blood sample using Lymphoprep™ (Palex Medical SA, Barcelona, Spain). After two washes in PBS, the cells obtained were resuspended in 200 mL binding buffer from the ANNEXIN V-FITC/7-AAD Kit (Beckman Coulter, Inc) in the presence of calcium.

Analysis of γδ and αβ T cells

To evaluate the functional analysis of γδ and αβ T cells - peripheral blood and intestinal tissue - we performed flow cytometry analysis with the following monoclonal antibodies: Anti-TCR PAN αβ- PE, anti-TCR PAN γδ- PE and FITC, CD19- PC7, CD56- PC7 and PE and CD4-PC7, CD3-PC5 and ECD, CD8-PC7 and FITC, CD5- FITC, CD45-ECD (Beckman Coulter). A total of 100000 events were acquired in a multiparameter Navios flow cytometer (Beckman Coulter) and later analyzed with Kaluza software.

Apoptosis evaluation

Apoptosis detection in peripheral blood was performed with ANNEXIN V-FITC/7-AAD Kit (Beckman Coulter), based on the binding properties of Annexin V to phosphatidylserine and on the specificity of 7-amino-actinomycin D (7-AAD) for DNA guanine-cytosine base pair, following instructions of the manufacturer. The gating strategy for T cell subsets in healthy controls *vs* patients with CD is shown in Supplementary Figure 1.

IL-7 in peripheral blood

The presence of serum IL-7 was measured by a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) (Quantikine® HS ELISA; R&D Systems, Minneapolis, MN, United States). According to the product information, the detection limit of the human IL-7 was 0.1 pg/mL with an assay range for serum samples of 0.25-16 pg/mL.

Gene expression of IL-7, IL-7 receptor α (CD127) and IL-2 receptor subunit γ (CD132) in tissues

The samples that were homogenized with 1 mL Trizol® Reagent (Ambion, Life Technologies, Austin, TX, United States) for RNA isolation following manufacturer's instructions. RNA concentration was measured using a GE NanoVue Spectrophotometer (GE Healthcare Life Sciences, Coventry, England).

A reverse transcription reaction was performed from 1 mg extracted RNA using the Thermo Scientific RevertAid H Minus First Strand cDNA Synthesis kit (Thermo Fisher Scientific, Waltham, MA, United States). Resulting cDNA was amplified and subjected to quantitative PCR (qPCR) in Gene Amp 5700 (PE Biosystems, Framingham, MA, United States). The primer sequences are shown in Supplementary Table 1. The relative expression of each gene was normalized against GADPH expression, used as reference standards.

Analysis of IL-7 and caspase-3 by Western blotting

Western blotting was performed with protein extracts from intestinal biopsies. Trizol/Guanidine Protein Purification Trizol® Reagent (Ambion, Life Technologies) and guanidine hydrochloride (Sigma) following the manufacturers' instructions, preserved in TNT buffer (20 mmol/L Tris-HCl, pH 7.5, 0.2 M NaCl, 1% Triton X-100) supplemented immediately before use with protease inhibitor cocktail tablets (10%) (Roche, Indianapolis, IN, United States). Protein concentration was determined using the Pierce BCA Protein Assay Reagent (Pierce, Rockford, IL, United States). The samples (20 µg protein) were subjected to electrophoresis on 12% polyacrylamide gels in the presence of sodium dodecyl sulfate. The proteins were transferred to a nitrocellulose membrane (Bio-Rad, Hercules, CA, United States), using the Bio-Rad Mini Protean II system in accordance with the conditions recommended by the commercial company.

The membranes were incubated with the corresponding primary antibody (anti IL-7 or anti-caspase-3) (Supplementary Table 2) in Tris-buffered saline (TBS) (20 mmol/L Tris-HCl pH 7.5 and 150 mmol/L NaCl) with 0.1% blocking agent, for 12 hours at 4 °C. Membranes were washed to remove excess primary antibody with TBS supplemented with 0.05% Tween, and then incubated with a 1/1000 dilution of the secondary antibody (Anti-Rabbit Ig-HRP; Sigma). Membranes were again washed, visualizing the positive bands by an image digitizer with the Enhanced Chemiluminescence reagent from Amersham (Buckinghamshire, England), and were quantified by densitometry using ImageJ software.

Statistical analysis

Mann-Whitney *U* test was used to compare differences between means of quantitative variables. Correlation studies using Spearman's correlation coefficient were performed to compare IL-7 gene expression, serum IL-7 levels, and T cell subsets. Data were analyzed using the Statistical Package for Social Sciences (SPSS 19.0; SPSS Inc., Chicago, IL, United States). Figures were performed using GraphPad Prism version 8.0.0 (GraphPad Software, San Diego, CA, United States).

RESULTS

Supplementary Table 3 shows the clinical characteristics of patients with CD (n = 80).

Cell number and percentage of apoptotic αβ and γδ T cell subsets in peripheral blood of patients with CD and healthy controls

There were no differences in the number of αβ T cells in patients with CD *vs* healthy subjects (Figure 1A). However, a significant decrease in γδ T cells in peripheral blood of patients with CD compared to control subjects was observed (Figure 1B). Percentages of αβ and γδ T cell subsets in the peripheral blood of patients with CD *vs* control healthy controls are shown in Supplementary Figure 2. We did not find any differences with respect to the absolute values.

Apoptosis of CD3+ CD56+ αβ, CD3+ CD56+ γδ, and CD3+ CD4+ γδ T cells was increased in patients with CD *vs* healthy subjects (Figure 1C and D). According to clinical scenarios, we found no significant differences in the number or apoptosis of αβ and γδ T cell subsets, except in CD3+ CD8+ γδ T cell number in active disease ($0.0057 \pm 0.0056 \times 10^6$/mL) *vs* remission ($0.0193 \pm 0.01991 \times 10^6$/mL) ($P$ = 0.008, Bonferroni test). There were also no differences among T cell subsets according to patients who were receiving treatment (n = 44, 55.0%) or were not treated at the time of the study (Supplementary Table 4).

Relationship between the number and apoptosis of αβ and γδ T cells

In Supplementary Figure 3, a significant relationship between γδ T cell subsets and their apoptosis is described in healthy subjects and patients with CD. There was no significant relationship between αβ T cells and their apoptosis in patients with CD.

In patients with CD, there was an inverse correlation (Spearman's r test) between T cell subsets and their apoptosis.

In healthy controls, there was an inverse correlation among both αβ and γδ T cell subsets and apoptosis except in CD3+CD56+ αβ T cells.

IL-7 gene and protein expression

Figure 2A shows IL-7 gene expression and levels of IL-7 cytokine in tissues of patients with CD *vs* healthy controls. Values were significantly higher in the CD group. In addition, there was a positive correlation between IL-7 gene expression and its tissue production (Figure 2B). However, there were no significant differences in serum IL-7 levels between patients and controls. Panel C shows the IL7 gene expression and Panel D shows the IL-7 protein levels

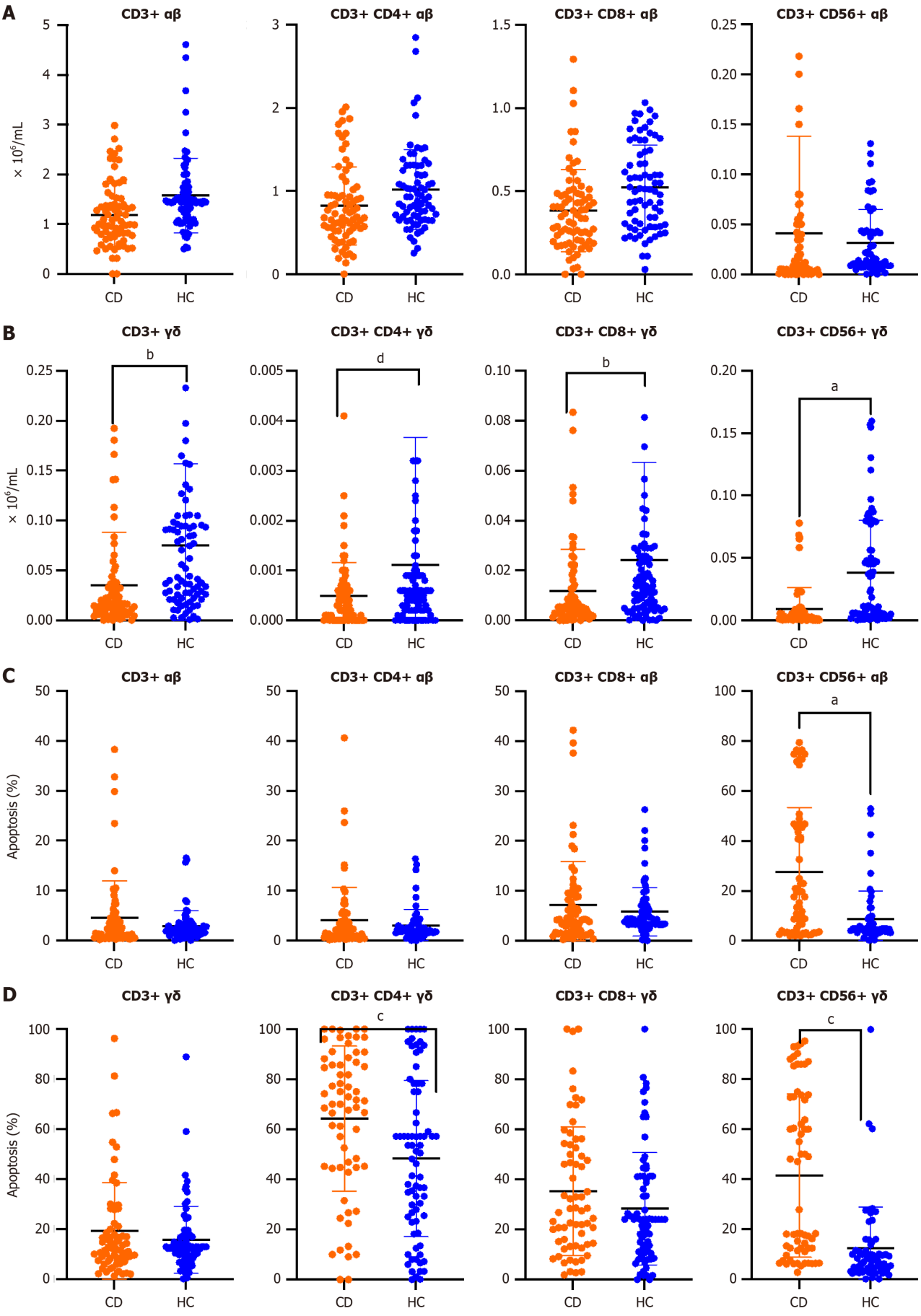


Figure 1 Numbers and apoptosis percentages of αβ and γδ T cell subsets in peripheral blood of patients with Crohn's disease (*n* = 80) *vs*

control healthy controls (*n* = 80). A and B: Numbers (means); C and D: Apoptosis percentages. Values are expressed as means (10^6/mL ×) and double T bars denote standard deviation. Mann-Whitney *U* test was used. [a]*P* < 0.05, [b]*P* < 0.01, [c]*P* < 0.001 and [d]*P* < 0.0001; IL: Interleukin.

(Figure 2C and D).

Expression of caspase-3 in tissues and its relationship with IL-7 and γδ T cells

Caspase-3 was significantly less expressed in the tissues of patients with CD when compared to controls (Figure 3A and B). Negative correlations were observed among expression of caspase-3 and frequencies of γδ T cells in the healthy control group (Figure 3C). We found no relationship between caspase-3 in tissues and αβ T cells in peripheral blood in healthy controls. This relationship also did not exist in αβ and γδ T cells in patients with CD.

Moreover, caspase-3 was negatively correlated with both IL-7 cytokine levels and IL-7 gene expression in tissues from patients with CD (Figure 3D).

We did not find significant differences in serum and tissue IL-7 cytokine, IL-7 gene expression and caspase-3 according to clinical scenarios and treatments.

Percentages of αβ and γδ T cells in tissues

Figure 4A and B show differences between tissue αβ and γδ T cells of patients with CD *vs* healthy controls, respectively. CD3+ γδ and CD3+CD8+ γδ T cells were significantly reduced in the tissue of patients with CD compared to controls. Serum IL-7 levels positively correlated with CD3+ γδ and CD3+ CD8+ γδ T cells in tissue of patients with CD (Figure 4C). There were no differences in the percentages of tissue T cells between patients with and without treatment. Panels D-G show flow cytometry imaging of αβ and γδ T cells (Figure 4D-G).

IL-7 receptor expression: IL-7 receptor α (CD127) and IL-2 receptor subunit γ (CD132)

Since there was no decrease in the gene expression of IL-7 and IL-7 cytokine in the tissues of patients with CD, we analyzed the expression of their receptors. As we can see in Figure 5A and B, the expression of IL-2 receptor subunit γ (CD132) was significantly lower in tissues of patients with CD compared to the control group. There was a significant positive correlation between the gene expression of IL7Rα (CD127) and serum IL-7 of all subjects (Figure 5C). In the case of CD132, the correlation, although positive, was not significant (Figure 5D). In four patients with CD, the gene expression of IL7R was studied. Three of the four patients were in remission and one in active disease. The patient with active disease was being treated with anti-TNF-α. No statistically differences were observed in the values of CD127 and CD132 between patients (Figure 5E).

DISCUSSION

The expression of the IL-7 gene and its receptors and the possible relationship with immunity and apoptosis of conventional and non-conventional αβ and γδ T cells, respectively, were studied in intestinal tissue and peripheral blood of patients with CD. In the present study, we confirmed the previously observed deficiency of γδ T cell subsets in peripheral blood from patients with CD[4]. Likewise, we observed the lowest mean frequency in CD3+ CD56+ γδ T cells. In addition, the apoptosis of CD3+ CD56+ αβ, CD3+ CD4+ γδ and CD3+ CD56+ γδ T cells was found to be higher in the patients with CD compared with controls. CD3+ CD56+ γδ T cells, share receptors with NK cells, have high cytotoxic activity and it was proposed to name them γδ NKT cells[21]. We previously described the importance of this subpopulation in immunity against infections and tumors. It has also been shown that both a deficiency in the number of cells and an increase in their apoptosis were related to a worse prognosis of sepsis and cancer disease[22-24]. Therefore, it is not surprising that, in the present study, patients with CD had lower CD3+ CD56+ γδ (γδ NKT cells) frequencies but with a higher degree of apoptosis.

The importance of IL-7 and its relationship in activation of T cells and specifically in γδ T cells was previously indicated in the introduction section. In the present work, we observed significantly higher tissue IL-7 gene expression in patients with CD compared to healthy controls. This increase was directly correlated to the production of the IL-7 cytokine. In addition, there were lower caspase-3 levels in patients with CD that were inversely correlated to expression of both tissue IL-7 gene and IL-7 protein. IL-7 prevented the spontaneous apoptosis of γδ intestinal intraepithelial lymphocytes in a murine model, although caspase activity was only slightly inhibited. This paper suggested that IL-7, stimulated by some unknown factor, could maintain enough γδ T cells by preventing their apoptosis[25]. These facts are in accordance with our results because the increase in the protein levels and gene expression of IL-7 together with the reduction of caspase-3 in the tissues of patients with CD suggests an attempt to maintain the number of γδ T cells. It has been observed that thymic stromal lymphopoietin may down-regulate caspase-3 expression through activation of the STAT3 pathway, thereby suppressing the apoptosis of γδ T cells[26].

However, this situation is not achieved, generating an immunosuppression (deficiency of γδ T cells) at mucosa-associated lymphoid tissue level that can be used by opportunistic pathogens that try to infect through the mucous membranes.

However, we observed discrepancies between tissue and serum levels of IL-7. The tissue levels of IL-7 increased to try to compensate for the γδ T cell deficit in patients with CD. However, serum IL-7 levels were similar in patients as

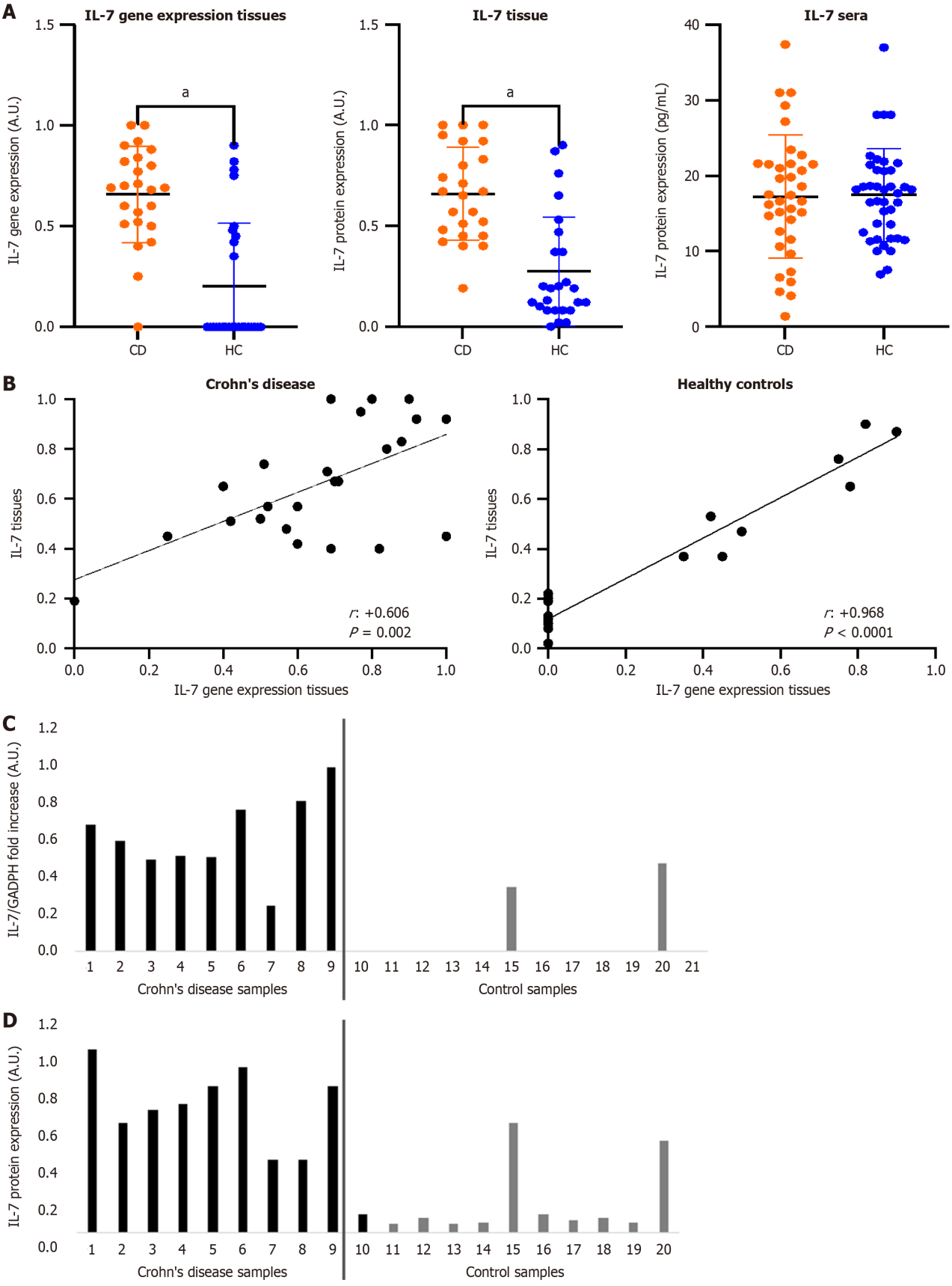


Figure 2 Interleukin 7 (IL-7) gene expression (retrotranscription-quantitative PCR) and IL-7 protein in tissues (Western blot analysis) and serum (enzyme-linked immunosorbent assay). A: Differences in interleukin 7 (IL-7) gene expression and IL-7 protein levels in tissues [arbitrary units (AU)] of patients with Crohn's disease (CD) (n = 25) *vs* healthy controls (HCs) (n = 25), and IL-7 in serum (pg/mL). Values are expressed as means, and double T bars denote standard deviation ($^aP < 0.05$). Mann-Whitney U test was used; B: Relationship of IL-7 gene expression and its levels in CD and HC tissues. Spearman's r test was used; C: IL-7 gene expression to perform retrotranscription-qPCR. The relative expression of each gene was normalized against glyceraldehyde-3-phosphate

dehydrogenase (GADPH) expression, used as reference standards; D: IL-7 protein expression. Western blot analysis was performed with protein extracts from intestinal biopsies. The loading control was done with anti-actin, 1:1000) (not shown). The visualized fragments were quantified by densitometry using the ImageJ software (National Institutes of Health, Bethesda, MD, United States).

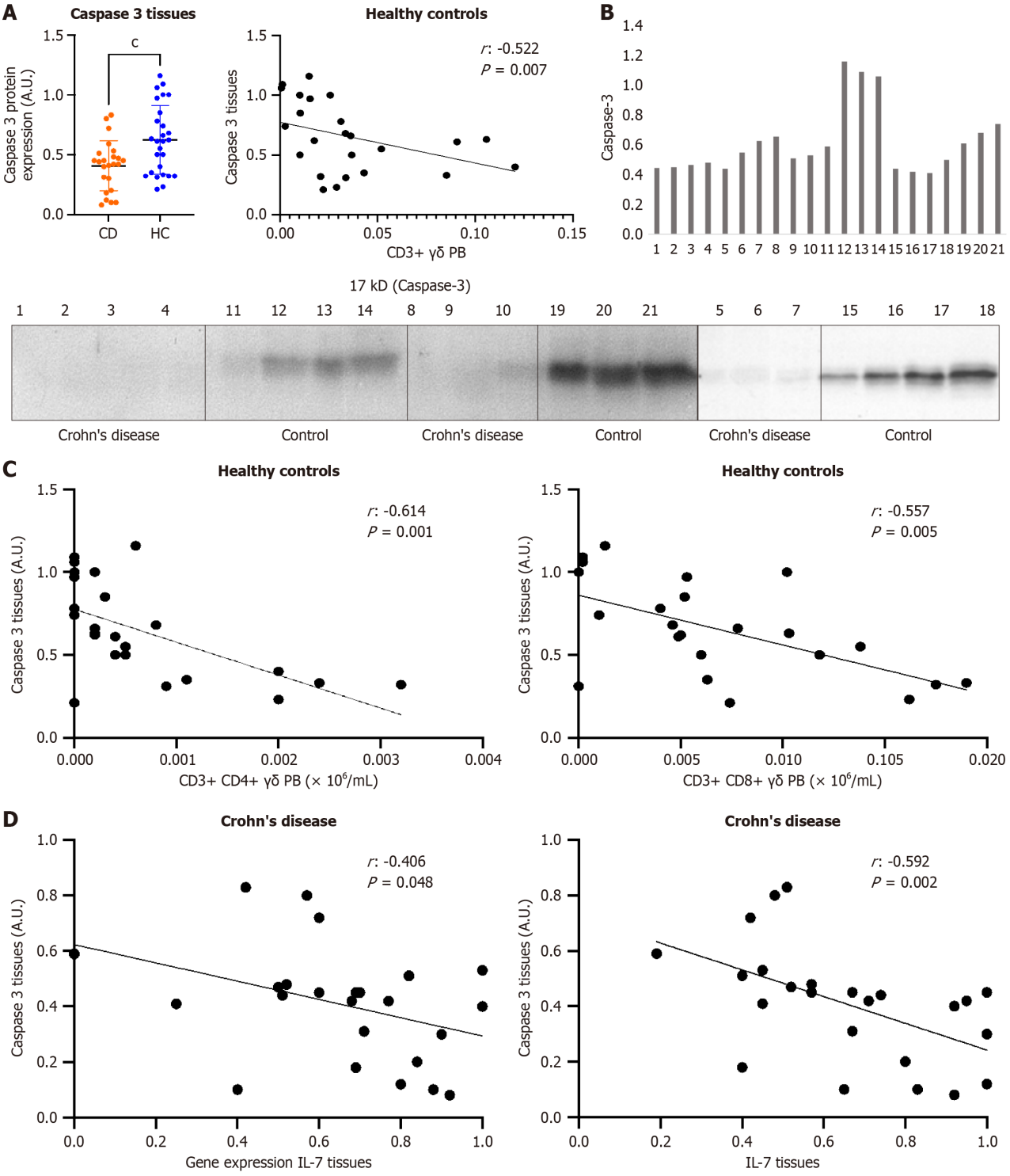


Figure 3 Caspase-3 protein in tissues (Western blot analysis). A: Differences of caspase 3 protein [arbitrary units (AU)] in tissues of patients with Crohn's disease (CD) (n = 24) *vs* healthy controls (n = 24). Values are expressed as means (× 10^6/mL), and double T bars denote standard deviation ($^cP < 0.001$). Mann-Whitney U test was used; B: Interleukin 7 (IL-7) protein expression. Western blot analysis was performed with protein extracts from intestinal biopsies. Loading control was done with anti-actin, 1:1000) (not shown). The visualized fragments were quantified by densitometry using ImageJ software (National Institutes of Health, Bethesda, MD, United States). Quantification of the expression band performed by ImageJ software; C: Significant relationship between γδ T cells subsets in peripheral blood and caspase-3 in tissues of healthy subjects. Spearman's r test was used; D: Relationship between caspase-3 protein in tissue and IL-7 gene expression and Il-7 protein in tissues of patients with CD (n = 25). Spearman's r test was used.

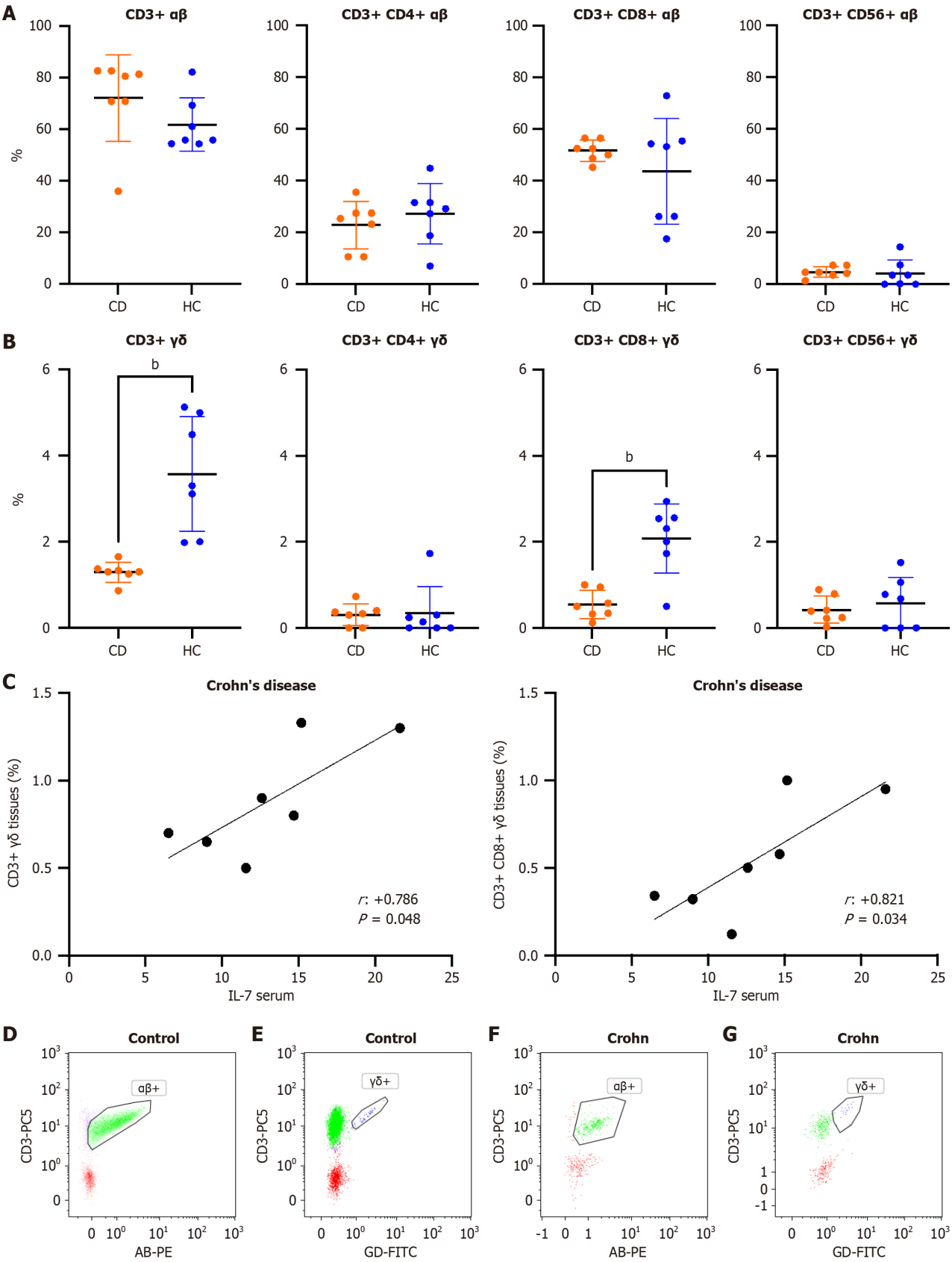
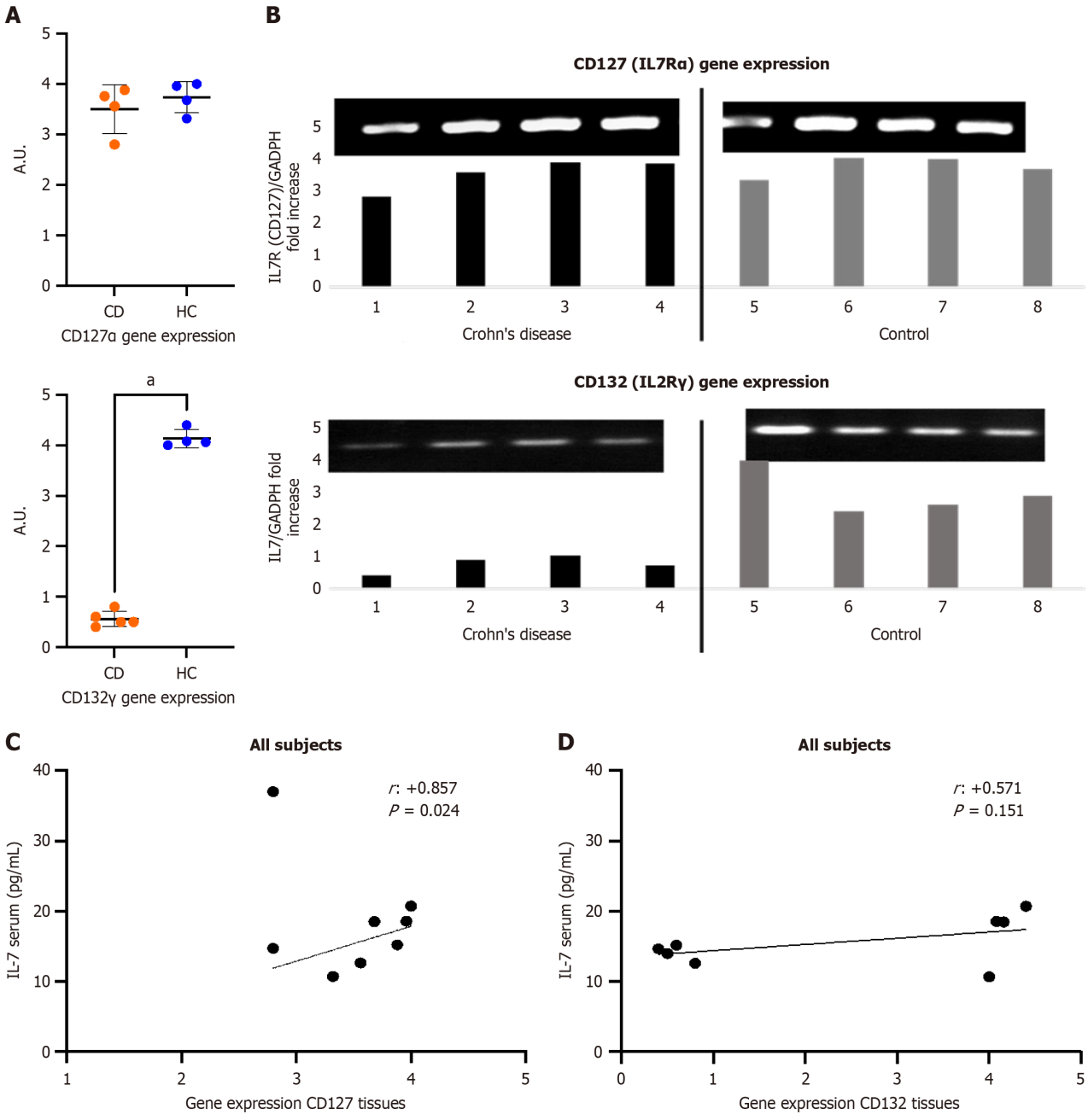


Figure 4 Frequency of T cells subset in tissues (flow cytometry). A and B: Comparison of αβ T cells (A) and γδT cells (B) in tissues from patients with Crohn's disease (CD) (n = 7) and healthy controls (n = 7). Values are expressed as percentages, and double T bars indicate standard deviation ($^bP < 0.01$). The Mann-Whitney U test was used; C: Relationship of interleukin 7 (IL-7) titers in peripheral blood with γδ T cells in tissues of patients with CD; D-G: Spearman's r test was used. Representative figures of αβ and γδ T cell subsets in tissues from healthy controls (D and E) and patients with CD (F and G) *vs* T cells were gated based on their low side scatter and high cluster of differentiation 45 (CD45) expression. After selection, we differentiated αβ+ (Panel D and F) and γδ+ (Panel E and G) T cell subsets in CD3 *vs* αβ/γδ dot plot.



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Characteristics of Crohn's disease patients (Gene expression IL7R)					
n	**Sex**	**Age**	**Case/control**	**Antivity**	**Montreal**
1	Female	53	Crohn	Active	A3-L1-B1
2	Male	42	Crohn	Remission	A3-L1-B1
3	Male	42	Crohn	Remission	A3-L3-B2
4	Male	22	Crohn	Remission	A2-L1-B1
5	Female	67	Control		
6	Female	59	Control		
7	Male	49	Control		
8	Female	49	Control		

Figure 5 Interleukin 7 receptor gene expression (retrotranscription-quantitative PCR). A: Comparation of interleukin 2 (IL-2) receptor subunit γ [cluster of differentiation (CD132)] gene expression of patients with Crohn's disease (CD) ($n = 4$) *vs* healthy controls (HCs) ($n = 4$); B: Gene expression of CD127 and CD132 to perform RT-PCR. The relative expression of each gene was normalized against glyceraldehyde-3-phosphate dehydrogenase (GADPH) expression, used as reference standards; C and D: Show the relationship of gene expression in tissues of CD127 and CD132 subunit with IL-7 serum titers of all subjects; E: Shows the characteristics of the subjects in whom the gene expression of IL-7 receptor has been analyzed. Values of CD127 and CD132 are expressed as arbitrary units (AU), and double T bars denote standard deviation. Mann-Whitney *U* test was used ($^aP < 0.05$). Correlations: Spearman's r test was used.

compared to healthy controls. This lack of increase in serum IL-7 Levels could be explained by a problem in the autocrine/paracrine effects of this cytokine. The cytokine may be being produced in large amounts but is being consumed by binding to the receptor, hence the importance of analyzing the IL-7 receptor.

The common gamma chain (γc or CD132 or IL-2 receptor subunit gamma or IL-2RG) integrates the IL-7 receptor heterodimer together with IL-7 receptor α (CD127). CD132 is a cytokine receptor subunit that, in addition to IL-7, is common to the other IL receptors: IL-2, IL-4, IL-9, IL-15 and IL-21. This receptor, expressed on most lymphocyte subsets, contributes to the proper functioning of the immune system, regulating development, proliferation, survival, and differentiation of immune cells[27]. Mutations in the gene encoding CD132 cause X-linked severe combined immunodeficiency in humans. Mechanisms of signaling and gene regulation of the related cytokines, provide information for immunodeficiency, autoimmunity, allergic diseases, and cancer[28]. Mutations in genes encoding cytokine receptors have been implicated in IBD, such as polymorphisms in IL-2, IL-21, IL-2RA, andIL-15RA[29]. According to the results shown in this study, the expression of the IL-2 receptor subunit γ (CD132) is greatly decreased in tissues of patients with CD. Gene expression levels of the IL-7 receptor α (CD127) were similar for controls and patients with CD. It is necessary to highlight that the integrity of the two subunits is necessary for the correct receptor functioning.

Therefore, we propose that significant increases in IL-7 gene expression, which also produces an increase of IL-7 production, and the low expression of caspase-3, which can lead a decrease in apoptosis, is insufficient to maintain homeostatic levels of γδ T cells in peripheral blood and tissues of patients with CD. It seems that a marked specific downregulation of the CD132 gene would inhibit the right effect of IL-7, leading to an immunodeficiency of γδ T cells that would favor superinfections or lack of elimination of some factors. This would allow the proliferation of etiological agents with consequent chronic inflammation. In a previous study, we described the relationship of an opportunistic fungus (microsporidia) with CD[30]. Recently, the destructive effects of microsporidium infection on epithelial barriers were demonstrated in a murine model by means of an increase of epithelium permeability and dysregulation of intestinal microbiota[31]. Based on the above evidence, we suggest that the infection by microsporidium, (either directly the pathogen or some protein thereof), could interferes with the expression of the receptor (CD132) as a mechanism to evade the host innate and adaptive immune responses.

CONCLUSION

There is an immunodeficiency of CD56+ αβ and γδ T cells in peripheral blood and tissues of patients with CD with increased apoptosis in peripheral blood. IL-7 gene expression and IL-7 protein levels in tissues of patients with CD were increased while the levels of caspase-3 in tissues were low. The increase in IL-7 and a lower expression of caspase-3 suggests a failure in the attempt to recover normal values of γδ T cells. The expression of IL-2 receptor subunit γ (CD132) is greatly decreased in the tissues of patients with CD. The lower gene expression of CD132 may be the cause of the decrease of γδ T cells. Further research would be necessary to determine the cause-effect of this correlation.

FOOTNOTES

Author contributions: Andreu-Ballester JC, Hurtado-Marcos C, and Cuéllar C contributed to the conception and design of the work; Gil-Borrás R contributed to the data collection and recruitment of patients; García-Ballesteros C and López-Chuliá F contributed to the flow cytometry analysis of T cell subsets and apoptosis; Pérez-Griera J and Cuéllar C contributed to interleukin 7 analysis and preparation of tissues for cytometry analysis; Hurtado-Marcos C, Ollero D and Izquierdo F contributed to PCR analysis of gene expression in tissues; Jiménez A contributed to tissue sampling; Andreu-Ballester JC contributed to the statistical method; Andreu-Ballester JC, Hurtado-Marcos C and Cuéllar C contributed to the interpretation of data for this work; Andreu-Ballester JC, Hurtado-Marcos C, Llombart-Cussac A, and Cuéllar C contributed to the final approval of the version to be submitted.

Institutional review board statement: This study was conducted following the recommendations of the Spanish Bioethics Committee, the Spanish legislation on Biomedical Research (Law 14/2007) and Personal Data Protection (Spanish Law 3/2018 and European Law UE676/2018). The anonymity of the subjects participating in the study has been ensured. The study was approved by the Ethics and Investigation Committee of the Arnau de Vilanova-Lliria University Hospital (Valencia, Spain).

Informed consent statement: All study participants or their legal guardian provided informed written consent about personal and medical data collection prior to study enrolment.

Conflict-of-interest statement: The authors have no conflicts of interest to declare.

Data sharing statement: No additional data is available.

STROBE statement: The authors have read the STROBE Statement—checklist of items, and the manuscript was prepared and revised according to the STROBE Statement—checklist of items.

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S-Editor: Li L
L-Editor: Filipodia
P-Editor: Zheng XM

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