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Different core-specific T cell subsets are expanded in chronic hepatitis C with advanced liver disease

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ABSTRACT

Chronic hepatitis C (CHC) is frequently related to liver fibrosis, and several studies have suggested that the immunological activity of HCV antigens contributes to hepatic damage. In the present study, among structural and non-structural HCV antigens, elevatedIL-1β, IL-6, IL-17 levels were secreted by PBMC cultures obtained from CHC patients following stimulation with core antigen. Moreover, the percentage of core-specific IL-6$^+$IL-17$^+$(CD4$^+$ and CD8$^+$) T cells was significantly higher in patients with worsehepatic lesions, determined on the Metavir scale. When compared with healthy subjects, the percentage of circulating Treg cells was elevated in CHC patients, mainly among those with advanced liver fibrosis. Nevertheless, in this last group of patients, the proportion of CD39$^+$ Treg subsets was very low. Finally, the percentage of senescent (CD57$^+$ CD28$^-$) and exhausted (PD-1$^+$CD28$^+$) core-specific T cells in CHC patients was also found to be a result of fibrotic hepatic status. In summary, imbalances between different core-specific T cell subsets are associated with liver fibrosis severity.

1. Introduction

It is estimated that 170 million people worldwide are infected by the hepatitis C virus (HCV). Due to the failure of the immune response to control and eliminate the virus during the acute phase, 60–80% of HCV-infected patients evolve to the chronic stage, where the significant number of patients who maintain persistent hepatic inflammation progress to liver fibrosis, cirrhosis and, ultimately, hepatocellular carcinoma [1–3].

HCV belongs to the genus *Hepacivirus* of the family *Flaviviridae,* with approximately 9.6-kb viral genome and is classified as a positive-strand RNA virus. The viral genome encodes immunogenic structural (core, E1 and E2) and non-structural (p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B) proteins. Some studies have demonstrated that sustained IFN-γ and IL-2 production by HCV-specific Th1 and T CD8$^+$ cells is involved in the control of viral replication [4,5]. In addition, disease resolution has been associated with the expansion of virus-specific IL-21-producing CD8$^+$ T cells associated with low frequency of dysfunctional HCV-specific T lymphocytes, identified by high expression of mucin-domain-containing-molecule- 3 (TIM-3) and programmed death 1 (PD-1) [6,7]. By contrast, failure to clear the virus and progression to the chronic phase are observed in patients with a high frequency of HCV-specific PD-1$^+$ Tim-3$^+$CD8$^+$T cells, which present reduced cell proliferation and low cytotoxic activity [8,9]. Furthermore, some studies have demonstrated that the inability of effector HCV-specific T cells to control viral replication is increased by the presence of expanded regulatory FoxP3$^+$CD25$^+$CD4$^+$ T cells (Tregs) in those patients [10,11]. Nevertheless, some authors have suggested that these Tregs play a protective role against hepatic damage in the chronic phase of infection by suppressing pro-fibrotic inflammatory response [12,13]. Given that these regulatory CD4$^+$ T lymphocytes present heterogeneous cell populations, studies evaluating the contribution of different Treg subsets, such those expressing CTLA-4 (CD152) and CD39, and hepatic fibrosis should be performed.

Although IL-21 is also a cytokine produced by Th17 cells, recent studies have suggested the involvement of these cells in promoting liver damage. In CHC, IL-17, IL-6 and transforming growth factor beta (TGF-β) have been found to be involved in liver fibrinogenesis [14].

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Moreover, a study we previously published [15] demonstrated that plasma levels of IL-17, IL-6 and IL-1β, as well as circulating IL-21$^-$ IFN-γ$^-$ IL-17$^+$ T cells, are associated with an elevated degree of liver fibrosis in CHC patients. On the other hand, in the same study, the frequency of polyclonally-activated IL-17$^+$ (CD4$^+$ and CD8$^+$) T cells able to produce IL-21and IFN-γ was related to low liver fibrosis. The objective of the present study was to analyze the antigenic specificity of different Th17-like cells in CHC patients with different degrees of liver stiffness.

2. Methodology

2.1. Patients and control subjects

For our study, 25 patients with chronic hepatitis C (CHC), genotype 1, were recruited from Gastroenterology & Hepatology Division of Internal Medicine Department at Gaffrée e Guinle Hospital/UNIRIO. The criteria for HCV infection was based on the presence of serum antibodies against HCV and detectable serum HCV RNA (real time PCR). Among CHC patients, 20/25 were naïve for HCV treatment, and 5/25 had previously failed anti-HCV therapy with pegIFN/ribavirin (at least 6 months prior to entering the study). Data regarding prior plasmatic levels of alanine transferase (ALT) and liver stiffness, determined using the Metavir scale, were obtained from medical records. In the present study, the Metavir-stage of CHC patients was: no (F0)/mild (F1) fibrosis (n = 12/25) and moderate (F2)/severe (F3) fibrosis (n = 13/25). As a control, 25 healthy age and sex-matched subjects, seronegative for HBV, HTLV and HIV, were also recruited to the study. We excluded individuals with a history of excessive alcohol intake and immunosuppressive drug use, as well as autoimmune diseases.

The study was approved by the Ethics Committee for Research on Human Subjects of the Federal University of the State of Rio de Janeiro (CAAE 30684514.7.0000.5258), and blood samples were only collected after obtaining written informed consent from each participant.

2.2. Cell cultures, plasmas and stimuli

Peripheral blood was collected in heparin-containing tubes (BD Vacutainer, Franklin Lakes, NY) and peripheral blood mononuclear cells (PBMC) were obtained by centrifugation on the Ficoll–Hypaque density gradient. Fresh viable PBMC (1×10^6/mL) were cultured in 24-well flat-bottomed microplates with 2 mL of RPMI 1640 medium supplemented with 2 mM of L-glutamine, 10% fetal calf serum (FCS), 20 U/mL of penicillin, 20 lg/mL of streptomycin and 20 mM of HEPES buffer. All reagents for culture medium were purchased from GIBCO (Carlsbad, CA, USA). To analyze the specific response to HCV, the PBMC cultures from CHC patients were stimulated for 6 days with 10 μg/mL of NS3/NS4, NS5 or core antigens (Austral Biologicals, CA, USA) plus 20 U/mL of human recombinant IL-2 (hrIL-2). Notably, hrIL-2 alone did not induce any detectable immune events assayed here (data not shown). The concentrations of HCV antigens were established in our laboratory as the optimum dose that induced T cell proliferation in chronically virus-infected patients. As a positive control, some cell cultures obtained from healthy patients were stimulated with phytohemaglutinin (PHA, 2 μg/mL) (Merck, N.J., USA). After 6 days, the supernatants were collected and the cytokine content analyzed by ELISA. In order to optimize the detection of intracellular cytokines by cytometer, the PBMC cultures, maintained for 6 days in the presence of core antigen, were kept for an additional 6 h in the presence of brefeldin A (10 μg/mL) (BD Biosciences, San Diego, CA, USA). BD. All cell cultures were maintained at 37 °C in a humidified 5% CO2 incubator.

2.3. Cytokine quantification

The quantification of cytokines from the supernatants collected from PHA-stimulated PBMC was performed using OptEIA ELISA kits (BD, Pharmigen, San Diego, CA), according to manufacturer's instructions. Briefly, each assay was performed using pairs of mAbs targeting human IL-1β, IL-6, IL-17, IL-21, IFN-γ and IL-10. The reaction was revealed with streptavidin-horseradish peroxidase, using 3.3′, 5.5′-tetramethyl-benzidine (TMB) as a substrate. Recombinant human cytokines, at concentrations ranging from 3.5 to 500 pg/mL, were used to construct standard curves.

Table 1
Characteristics of chronically HCV-infected patients[a].

	Patients (n = 25)
Age in years [mean (range)]	52.3 (33–66)
Time in years since HCV diagnosis [mean (range)]	12.7 (1–23)
Gender, female/male (n)	18/7
Treatment	
Naïve (n)	20
Failed (n)	05
ALT (U/L) [mean ± SD]	
Naïve	41.8 ± 24.2
Failed	49.6 ± 25.4
Mean viral load (in log copies/mL ± SD	
Naïve	4.73 ± 1.8
Failed	3.94 ± 2.2

[a] Indivudulas chronically infected with hepatitis C virus (HCV) genotype 1 (n = 25) and detectable plasma viral load were enrolled in the study aiming to evaluate some immune parameters and correlate them with clinical markers of liver function and fibrosis. While 20 were naïve for HCV therapy, 5 had previously failed pegIFN/Ribavine treatment. Alanine transferase (ALT) levels were measured at the same time as blood sampling to analyze immune parameters.

2.4. Flow cytometry analysis

Mouse anti-human monoclonal antibodies (mAbs) for CD3-PE, CD8-FITC, CD4-FITC, CD4-FoxPe-APC, CD25-PECy7, CD152-PE, CD39-PECy5/PE, CD57-APC, CD28-PECy7, PD-1-APC, and all isotype control antibodies were purchased from BD Bioscience (San Diego, CA, USA). Briefly, 200 μL of PBMC (1×10^5 cells) were incubated with various combinations of the aforementioned mAbs for 30 min at room temperature in the dark, according to manufacturer'sinstructions. The cells were washed with phosphate-buffered saline containing 1% bovine serum albumin and permeabilization was performed using incubating cells with Cytofix/Cytoperm solution (BD Pharmigen, San Diego, CA) at 4 °C for 20 min. The mAbs for intracellular staining (anti-IL-6-PE-Cy7, IL-17-APC) were added and then incubated for 30 min at 4 °C. The cells were acquired at Accuri C6 (Accuri™, Ann Arbor, MI, USA) and analyzed using Cflow (Accuri™, Ann Arbor, MI, USA). Isotype control antibodies and single-stained samples were used to periodically check the settings and gates on the flow cytometer. After acquisition of 200,000 events, lymphocytes were gated based on forward- and side-scatter properties after the exclusion of dead cells using propidium iodide and doublets. Additionally, the gated cells were negative for CD14 marker.

2.5. Statistical analysis

Statistical analysis was carried out using Prism 5.0 software (GraphPad Software). The data normalization before comparing the findings obtained from control and CHC cultures, as well as PBMC cultures, was performed. The nonparametric Mann-Whitney *U* test and the Student's *t*-test were applied to determine whether the two groups were statistically different for nonparametric and parametric variables, respectively. Significance in all experiments was defined as $p < 0.05$.

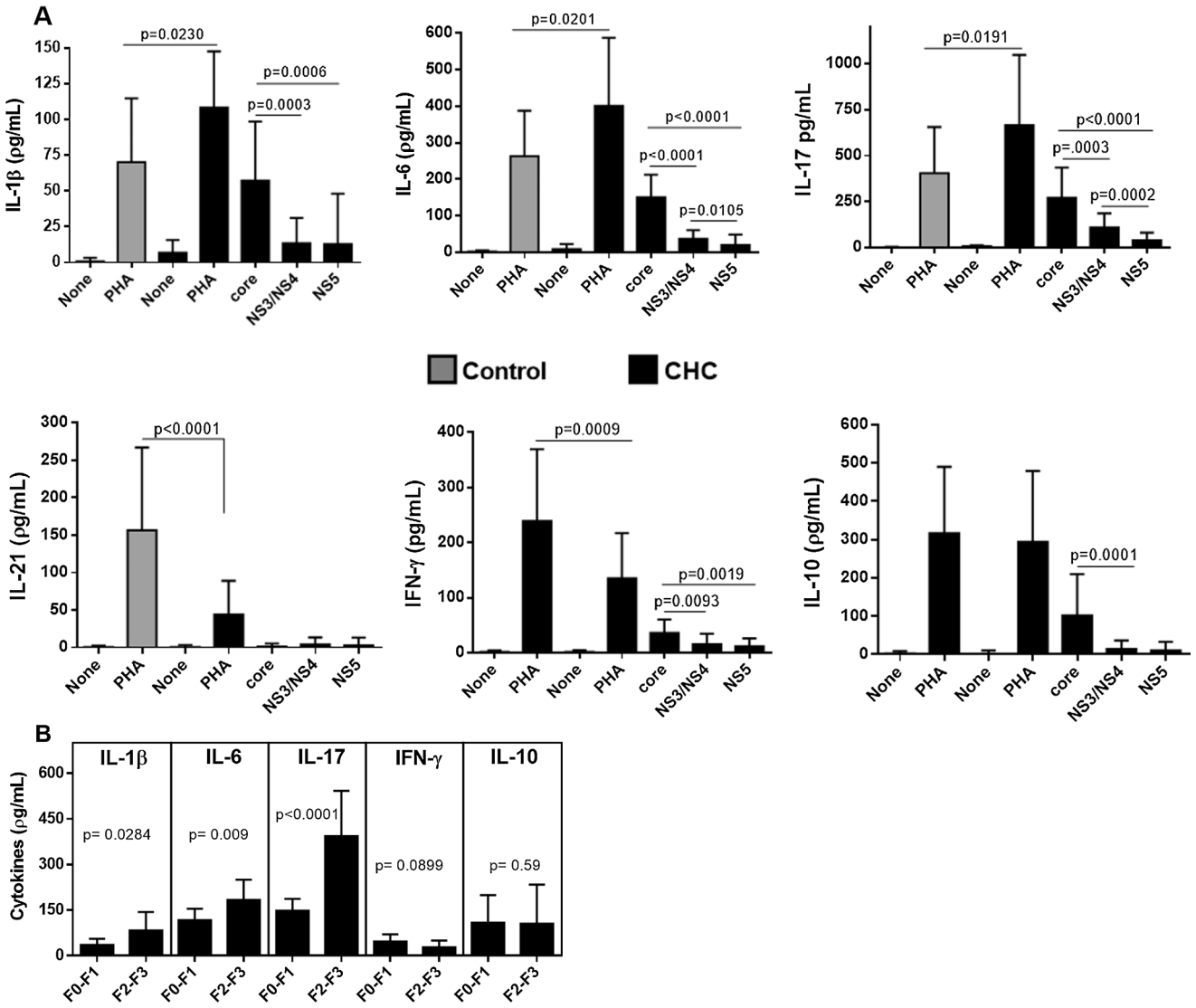

Fig. 1. The production of cytokines by PBMC cells from patients with chronic hepatitis C (CHC). PBMC (1×10^6/mL) from chronically HCV-infected patients (n = 25) and healthy individuals (control, n = 25) were maintained for 6 days with PHA (1 μg/mL) or in the presence of HCV antigens [core (10 μg/mL), NS3/NS4 (10 μg/mL) or NS4 (10 μg/mL)] in combination with rhIL-2 (20 U/mL). As a negative control, the cells were maintained in medium alone. In (A), the cytokine levels were determined by ELISA, and in (B), the cytokine levels induced by core protein were stratified according the Metavir scale (F0-F1 = 12 and F2-F3 = 13). The mean values were compared and the *p* values are shown in each graph.

3. Results

3.1. Characteristics of CHC patients, cytokine dosage and its relationship with clinical parameters

For our study, viremic CHC patients (18 women and 7 men), free of HCV medication at the time of blood sampling, were recruited and T-cell response to different HCV antigens was analyzed and correlated with hepatic stiffness. Although 5/25 CHC patients had failed prior pegIFN/ribavirin therapy, no difference was observed either in terms of ALT levels, viremia or time since HCV diagnosis (Table 1).

Here, in the presence of PHA, the T cells from CHC patients produced higher IL-1β ($p = 0.0230$), IL-6 ($p = 0.0201$) and IL-17 ($p = 0.0191$) levels than the control cultures (Fig. 1A). By contrast, lower IL-21 ($p < 0.0001$) and IFN-γ($p = 0.0009$) levels were released inpatients by these polyclonally-activated cell cultures than in healthy subjects (Fig. 1A). No difference was observed regarding IL-10 production between the two groups in response to PHA (Fig. 1A). Concerning HCV antigens, among CHC patients, the secretion of IL-1β, IL-6, IL-17, IFN-γ and IL-10 was significantly higher in core-stimulated PBMC cultures than those maintained in the presence of non-structural proteins (NS3/NS4 and NS5) (Fig. 1A). Nevertheless, in comparison with non-stimulated (medium alone) cell cultures, NS3/NS4 antigens significantly induced IL-6 ($p = 0.037$), IL-17($p < 0.0001$), IFN-γ ($p = 0.0020$) and IL-10 ($p = 0.0313$) production. The addition of NS5 also increased the release of IL-17 ($p = 0.0007$), IFN-γ ($p = 0.0006$) and IL-10 ($p = 0.0156$) (Fig. 1A). More importantly, among HCV antigens, higher IL-1β, IL-6, IL-17 cytokine levels were secreted by core-stimulated PBMC cultures from CHC patients with moderate (F2)/severe (F3) fibrosis, than in those with no (F0)/mild (F1) fibrosis (Fig. 1B). Notably, as also observed in our previous study [15], here, IL-17 and IL-6 levels produced by polyclonally-activated T cells were also directly associated with liver fibrosis (data not shown). The plasma concentration of ALT was normal in the great majority of CHC patients and, here, no correlations were observed between the cytokines analyzed and the levels of this hepatic transaminase (data not shown).

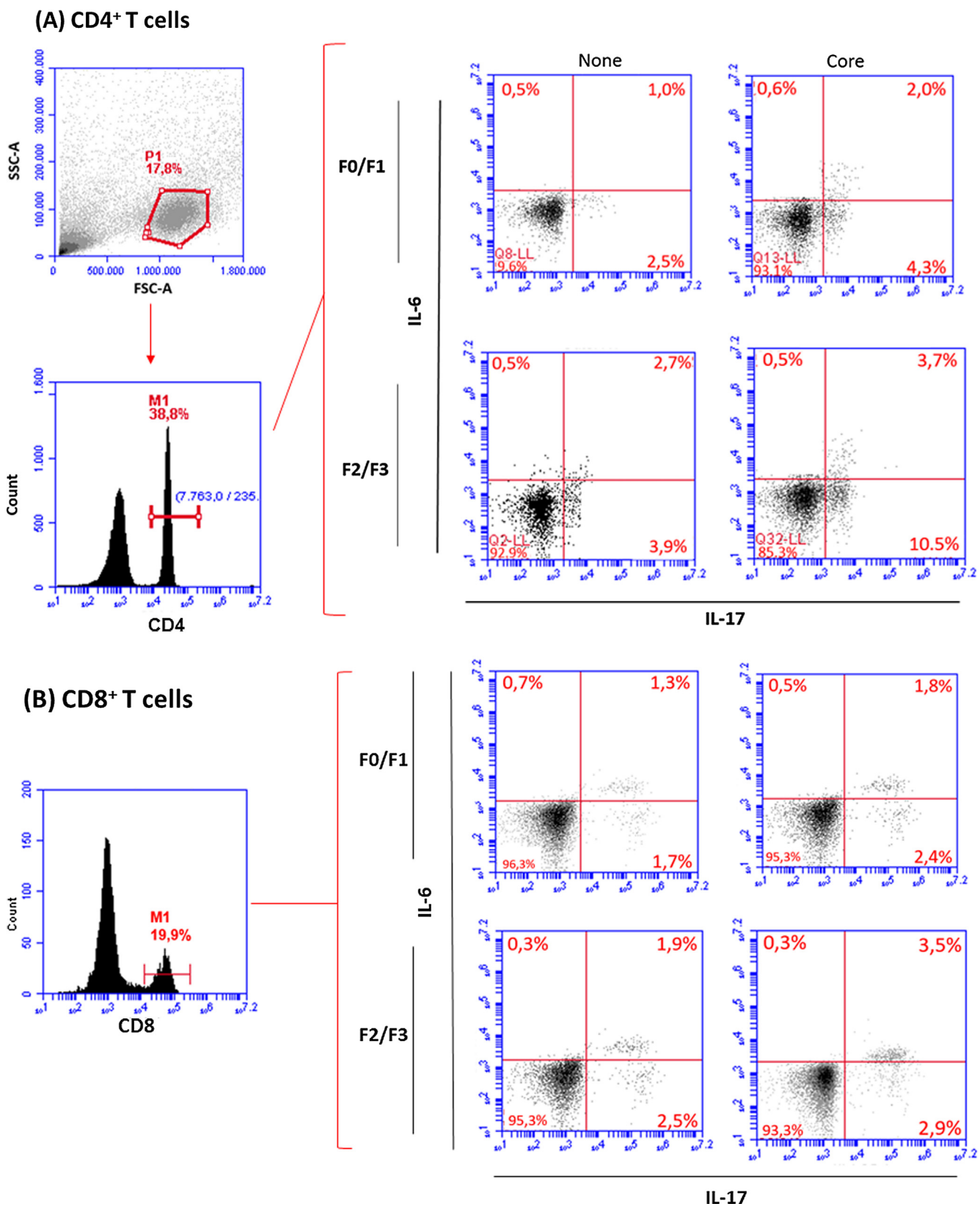

Fig. 2. The analysis of cytokine-secreting T cells induced by core protein due to hepatic lesions. The proportion of $CD4^+$ and $CD8^+$ T cells that produced IL-16 and IL-17 was determined in chronically HCV-infected patients with no (F0)/mild (F1) (n = 12) and moderate (F2)/severe (F3) (n = 13) liver fibrosis after activating PBMC cultures with core protein (10 μg/mL) plus rhIL-2 (20 U/mL) for 6 days. The dot-plots presenting identification of $CD4^+$ (A) and $CD8^+$ (B) T cells from CHC capable of producing IL-17 and IL-6 are demonstrated. In (C), the mean values were compared and the *p* values are shown in each graph.

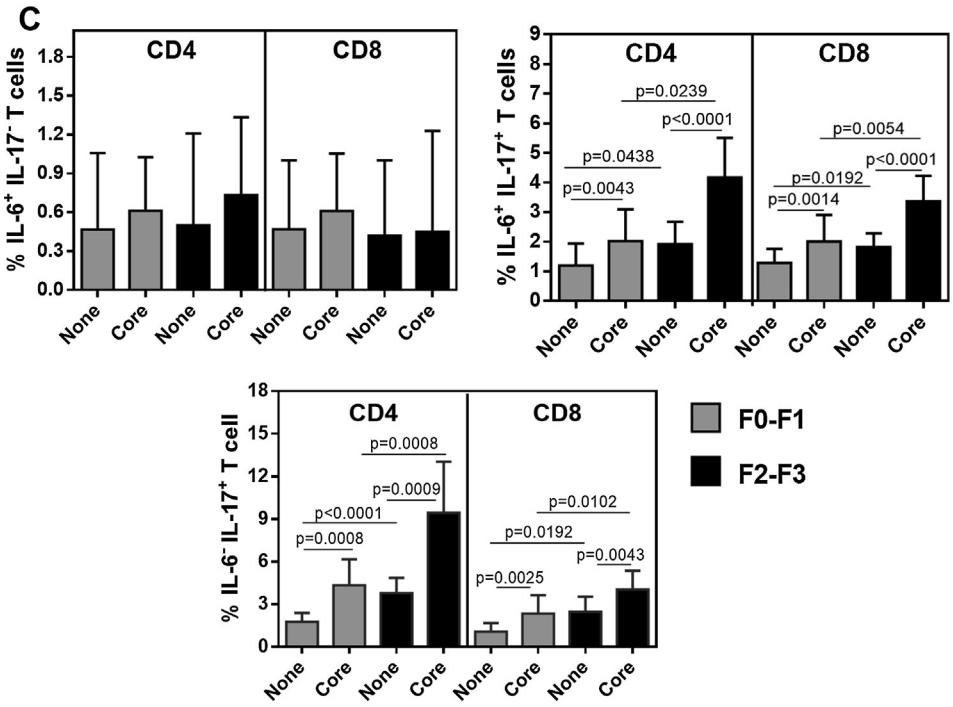

Fig. 2. (*continued*)

3.2. Core-specific Th17 and Tc-17 subsets are expanded in CHC patients with advanced liver disease

Taking into consideration the *p* values, both IL-6 and IL-17 were the cytokines released by core-stimulated PBMC cultures most strongly associated with liver fibrosis. Through flow cytometry analyses, and taking into consideration the gating strategy in the Fig. 2A, the percentage of both IL-17$^+$ and dual IL-17$^+$IL-6$^+$ (CD4$^+$ and CD8$^+$) T cell subsets were significantly higher in CHC patients with advanced liver stiffness, and the proportion of these Th17-like subsets was elevated following the addition of core protein (Fig. 2B). The frequency of other IL-17$^+$ T cell subsets able to produce IL-1β, IFN-γ and IL-10 was very low and they were not correlated with hepatic lesions (data not shown). Additionally, no T cell phenotypes were correlated with ALT levels.

3.3. Low CD39$^+$ Tregfrequency and a high proportion of dysfunctional T cells were observed in CHC patients with advanced liver fibrosis

CHC infection has been associated with high frequency of FoxP3$^+$CD25$^+$CD4$^+$ T (Treg) cells [10,11], and following the representative flow cytometry dot-plots and histograms showing the gating strategy for Treg subsets (Fig. 3A), these cells were expanded in chronically HCV-infected patients, mainly in patients with worse liver outcomes (Fig. 3B). Furthermore, we also observed a higher frequency of CD152$^+$Treg subsets among CHC patients (Fig. 3D). By contrast, the percentage of CD39$^+$Treg subsets was dramatically reduced in CHC patients with a higher Metavir score (Fig. 3C). The addition of core antigen (Fig. 3B–D), and non-structural (NS3/NS4 and NS5) proteins (data not shown), did not alter the proportion of these Treg subpopulations. It is important to mention that similar Treg subset imbalances were observed in freshly purified PBMC cells from CHC patients (data not shown).

Chronic HCV infection is associated with a high frequency of dysfunctional T cells directed against the virus [6,7]. Here, taking into consideration the representative dot-plots showing identification of exhausted (PD-1$^+$CD28$^+$) and senescent (CD57$^+$CD28$^-$) CD4 (Fig. 4A) and CD8 (Fig. 4C) T cells, the percentage of PD-1$^+$ CD28$^+$ (CD4$^+$ and CD8$^+$) T cells was significantly higher in both F0/F1 ($p = 0.0016$ for CD4 and $p = 0.0004$ for CD8) and, mainly, F2/F3 ($p < 0.0001$ for CD4 and CD8) patients as compared with control samples (Fig. 4B). Notably, core protein up-regulated the proportion these cells in PBMC cultures from CHC patients (Fig. 4B). Furthermore, in comparison with the healthy cell cultures, an elevated percentage of circulating CD57$^+$CD28$^-$ (CD4$^+$ and CD8$^+$) T cells was observed in patients with reduced ($p = 0.0202$ for CD4 and $p = 0.0328$ for CD8) and elevated liver fibrosis ($p < 0.0001$ for CD4 and $p = 0.0061$ for CD8) (Fig. 4D). Moreover, in cell cultures from F2/F3 group, the proportion of this senescent T cell additionally increased after the addition of core antigen (Fig. 4D). Significantly, an elevated frequency of these exhausted and senescent T cells was also detected in CHC-derived PBMC before culturing (data not shown).

4. Discussion

The immune events following HCV infection are complex and the mechanisms involved in virus clearance and disease resolution, as well as chronicity and liver damage, are not completely understood. In the present study, we demonstrated that an imbalance between different core-specific T cell phenotypes could be involved in hepatic fibrosis in CHC patients.

Some studies have demonstrated that the cytokine profile of HCV-specific T cells affects the risk of liver fibrosis, cirrhosis and subsequently, hepatocellular carcinoma [14,16]. Here, in comparison with non-structural antigens (NS3/NS4 and NS5), elevated levels of Th17-related cytokines were detected in core-stimulated cell cultures from CHC patients. Further, higher IL-1β, IL-6 and IL-17 levels in response to core protein were dosed in cell cultures of patients with advanced liver fibrosis. No difference was observed in IFN-γ and IL-10 production, and IL-21 was almost undetectable.

Although IL-21 is produced by classical Th17 cells, some activated T cells release IL-21 regardless of IL-17 [17], and a high IL-21/IL-17 ratio in response to HCV appears to determine infection resolution [7,14]. In our previous study, a higher proportion of IL-17$^+$IL-21$^-$ (CD4 and CD8) T cells associated with a very low percentage of IL-21-secreting T cell subsets following brief activation with PMA and inomycin was associated with liver stiffness [15]. Interestingly, in the present study,

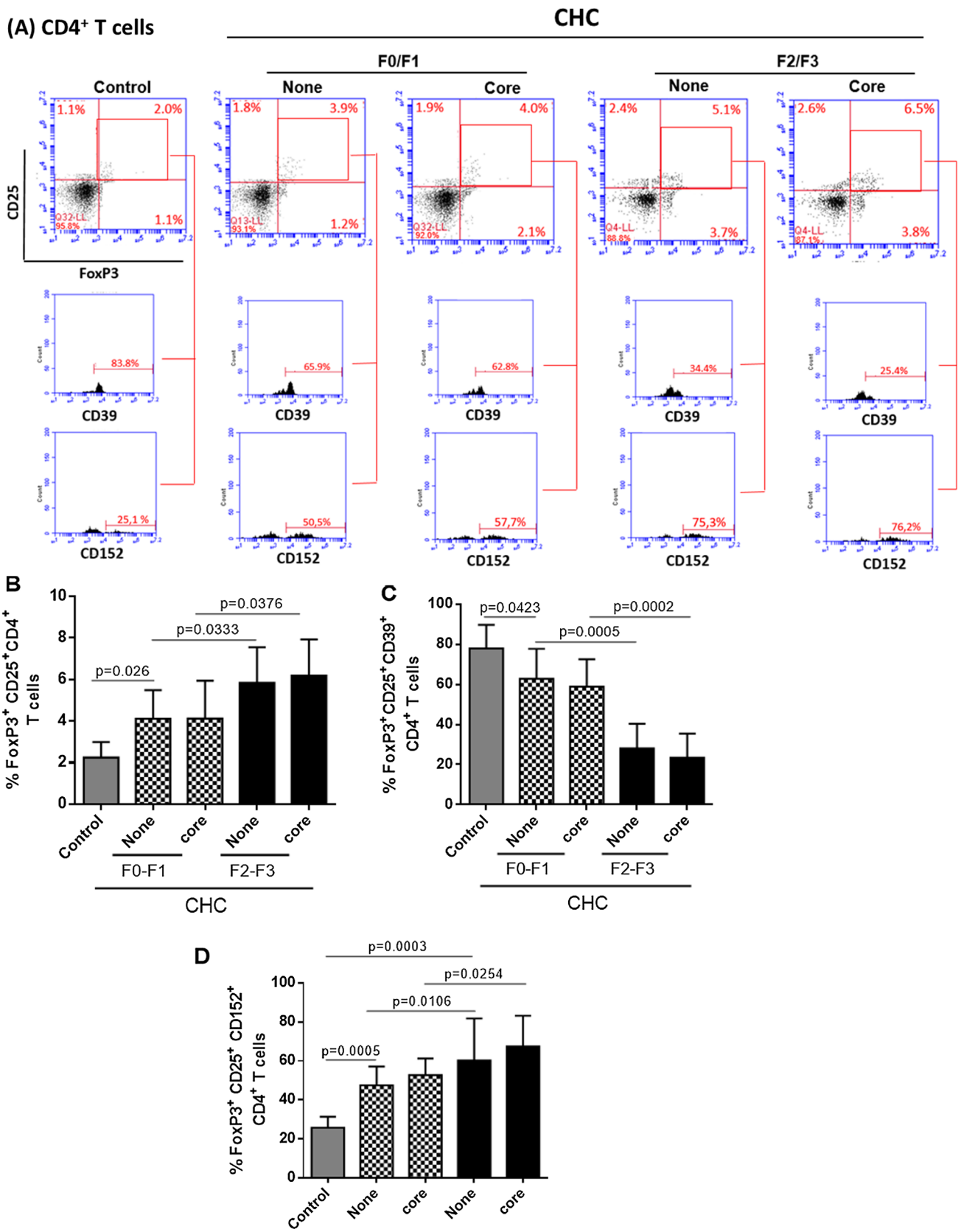

Fig. 3. The percentage of core-specific Tregs in CHC patients with different degrees of liver fibrosis. The mean percentage of whole Tregs (B), and $CD39^+$ (C) or $CD152^+$ (D) Treg subsets was determined in CHC patients with different Metavir scores [F0-F1 (n = 12) and F2-F3 (n = 13)]. The PBMC were maintained in the presence of medium alone (none) or with core antigen plus rhIL-2 for 6 days. As a control, the same T cell subset analyses were performed in non-stimulated PBMC cultures from healthy subjects. Representative dot-plots and histograms showing identification $FoxP3^+CD25^+CD4^+$ T cells positives for CD39 or CD152, are demonstrated in panel (A). The mean values were compared and the *p* values are shown in each graph.

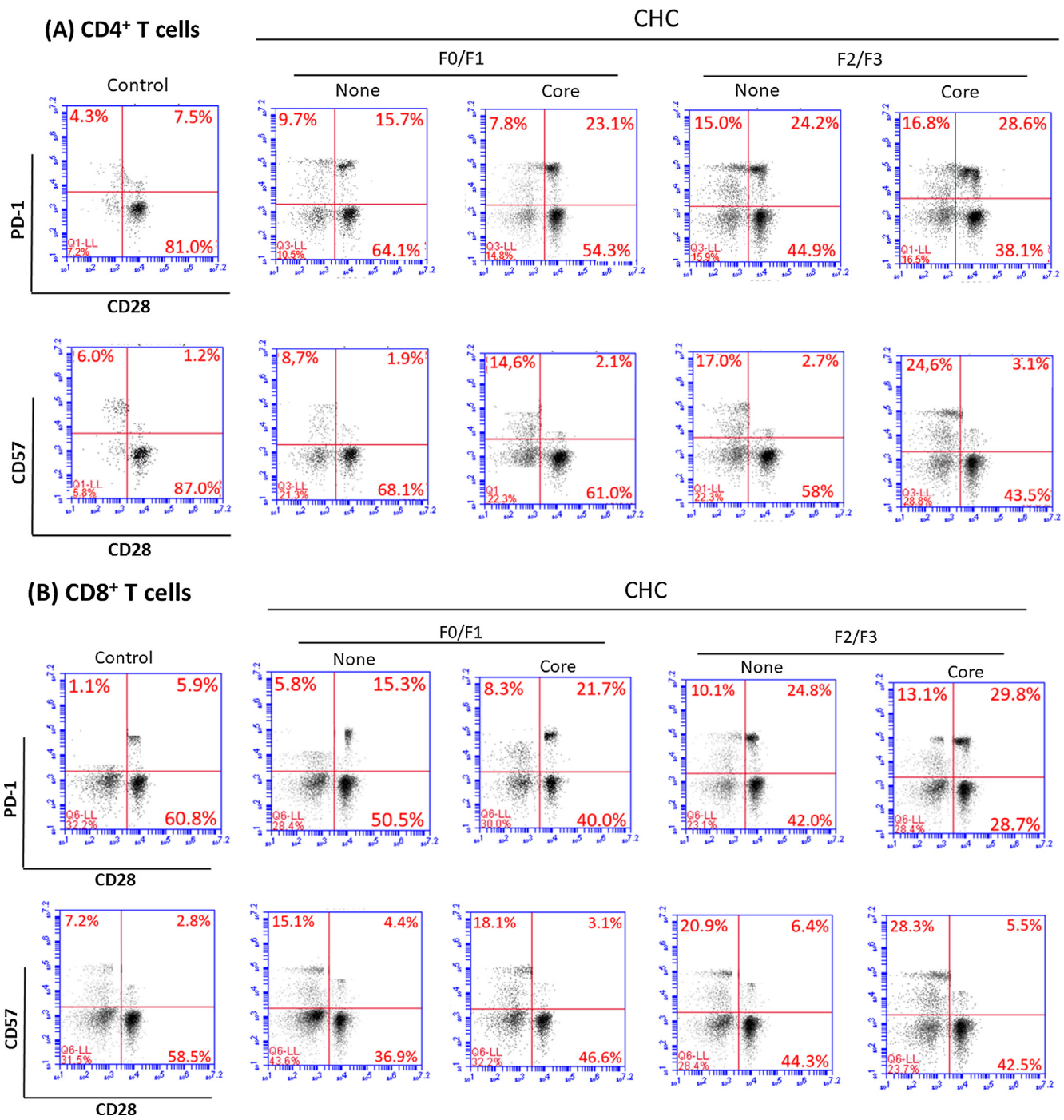

Fig. 4. The percentage of exhausted and senescent core-specific T cells in CHC patients in function of liver fibrosis. The percentage of PD-1$^+$ (C) and CD57$^+$CD28$^-$ (D) into CD4$^+$ and CD8$^+$ T cells compartment was determined after maintaining PBMC cultures, obtained from CHC patients with different Metavir scores [F0-F1 (n = 12) and F2-F3 (n-13)], in medium alone (none) or in the presence of core antigen (10 μg/mL) plus rhIL-2 (20 U/mL) for 6 days. As a control, the same T cell subsets were analyzed in non-stimulated PBMC cultures obtained from healthy subjects. Representative dot-plots showing identification of CD4$^+$ and CD8$^+$ T cells co-expression CD28 with along PD-1 and CD57 are demonstrated on panels A and B, respectively. The mean values were compared and the *p* values are shown in each graph.

both single IL-17$^+$ or dual IL-17$^+$IL-6$^+$ (CD4$^+$ and CD8$^+$) T cells were associated with an elevated Metavir score. More importantly, core protein up-regulated both Th17-like phenotypes *in vitro*. These results suggest that core-specific Th17 cell subsets may contribute to liver stiffness. In agreement with the present study, some studies have demonstrated the ability of IL-17 to trigger liver profibrotic pathways [14]. In this scenario, stellate and kupffer cells express IL-17 receptors, and respond to this cytokine by producing type I collagen and TGF-β, respectively [14,18,19]. Moreover, dominance of Th17/IL-17 axis during HCV infection also amplifies pro-fibrotic events by promoting intense hepatic recruitment of activated neutrophils and monocytes [14,18].

In addition to IL-17 production, the Th17-mediated immune response is also accompanied by the production of elevated amounts of other potent pro-inflammatory cytokines, such as IL-1β and IL-6 [20], and IL-6, which have been linked to liver damage during HCV infection [21,22]. Shah et al. [22] suggested that IL-6 levels are predictive of liver fibrosis. Furthermore, IL-6 and IL-1β favor expansion of human

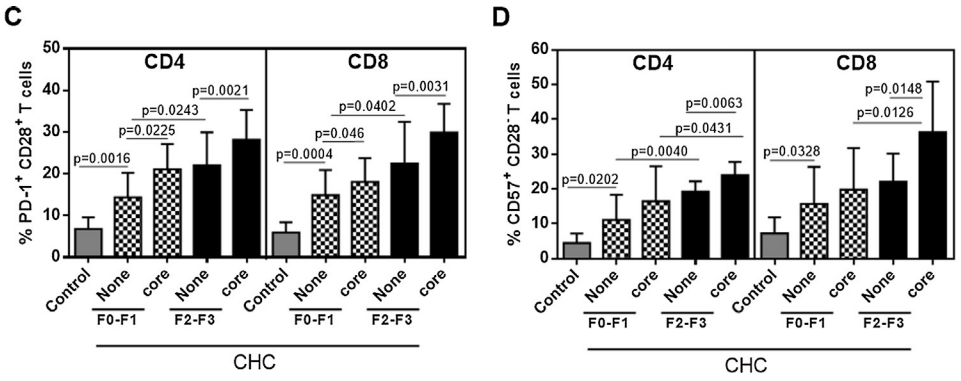

Fig. 4. (*continued*)

pathogenic Th17 phenotypes [14,20,23–25]. Collectively, our study demonstrated a relationship between the expansion of particular core-specific IL-6-secreting Th17-like subsets and liver fibrosis for the first time. Our results should be validated in a larger cohort of CHC patients, mainly in the context of a new potent anti-HCV therapy protease based on protease inhibitors.

It is known that core protein, through its ability to bind to toll-like receptor 2 (TLR2), may favor liver fibrosis by activating vascular endothelial growth factor (VEGF) expression in hepatic stellate cells [26,27]. Moreover, core antigen, by signaling through TLR2 in dendritic cells, may favor Th17 differentiation [17]. In autoimmune disease settings, such as multiple sclerosis, pathogenic Th17 cells express high levels of functional TLR2 [28,29]. There is a possibility that core protein, by signaling via TLR2, directly favors the expansion of hepatotoxic IL-6$^+$ Th17-like phenotypes during CHC.

With regard to IL-1β, their levels dosed in core-induced PBMC cultures were associated with liver stiffness, but no relationship was observed in terms of the percentage of IL-1β$^+$ T cells, which suggests that other immune cells should respond to these HCV antigens. As core proteins can trigger inflammatory cell activation via TLR2, the source of IL-1β in core-stimulated PBMC cultures could be derived from innate immune cells, such as monocytes [30].

It is expected that Treg frequency increases to control ongoing inflammatory processes, and some studies have documented an elevation of circulating FoxP3$^+$ CD25$^+$CD4$^+$Tregs during HCV infection [10,11]. This phenomenon would be potentiated in the liver since resident cells naturally favor immune tolerance [31]. In the present study, the percentage of circulating Tregs, and Treg subsets expressing CD152, were indeed elevated in CHC patients, mainly in patients with advanced liver fibrosis. Nevertheless, the frequency of CD39$^+$Treg subsets was dramatically reduced in patients with a high Metavir score and although the addition of HCV antigens did not modify the proportion of either Treg subsets, a lower percentage of CD39$^+$Tregs may affect disease outcomes. CD39 works together with CD73 to regulate immune response through conversion of ADP/ATP to adenosine [32–34], a nucleoside with potent imunossupressive action on effector T cells expressing A2A receptors [34–36]. Thus, CD39 expression helps to identify more functional Tregs [33,36–38], and deficiency in this Treg subset has been reported in different autoimmune disorder settings [39]. Therefore, although preliminary, suboptimal functioning of Treg subsets may contribute to inflammatory-mediated liver damage in CHC.

Finally, in the present study, the percentage of T cells expressing exhausted (PD-1$^+$) and senescent (CD57$^+$CD28$^-$) T cells was higher in CHC patients, mainly among those with high Matevir scores. Core protein elevated the frequency of both T cell subsets in CHC patients with advanced hepatic stiffness, as well as up-regulating PD-1$^+$ T cells in patients with better liver conditions (F0/F1). PD-1 is a member of the CD8/CTLA-4 family that downregulates T-cell receptor (TCR) signaling and, consequently, promotes decreased cytokine production and reduced T-cell proliferation [40,41]. An elegant study by Kared et al. [7] demonstrated that a high frequency of PD-1$^+$T cells during the acute phase of HCV infection increases the risk of progressing to the chronic phase, which was associated with an inability by HCV-specific PD-1$^+$ T cells to clear the virus. Additionally, the accumulation of senescent T cells has been observed in CHC patients [42]. These senescent T cells, induced by repeated antigenic stimulation, may be identified by the expression of CD57 associated with an absence of CD28 molecules [42]. Classically, these T cells have critically shortened telomeres [43], which impairs their ability to proliferate [44]. According to Horare et al. [45], in HCV infection, CD4$^+$T lymphocyte telomere length is closely associated with fibrosis, inflammation, prolonged prothrombin time and alterations inbilirubin levels. In the present study, a higher proportion of CD57$^+$CD28$^-$ T cells was detected in PBMC cell cultures from CHC patients with advanced liver stiffness, mainly after the addition of core protein. Therefore, as several CD57$^+$CD28$^-$T cells produce pro-inflammatory cytokines, such as TNF-α, the ability of core protein to expand this T cell subset may contribute to liver fibrosis.

In conclusion, although preliminary, our results suggest that an imbalance in different Th17 and Treg subsets, associated with the expansion of the senescent/dysfunctional T cell compartment may contribute to fibrotic hepatic status in chronically infected patients, revealing the deleterious role played by core antigen in favoring this immunological homeostasis breakdown.

5. Conflict of interest statement

All authors declare that there are no conflicts of interest.

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