Journal of Interventional Cardiac Electrophysiology (2019) 54:257–265
https://doi.org/10.1007/s10840-018-0491-3

Impact of cardiac resynchronization therapy on circulating IL-17 producing cells in patients with advanced heart failure

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Received: 28 June 2018 / Accepted: 14 November 2018 / Published online: 27 November 2018

Abstract

Purpose IL-17-producing T cells have been implicated in the inflammatory milieu of chronic heart failure (CHF), which implies a dismal prognosis in affected patients. The aim of this study was to evaluate the impact of cardiac resynchronization therapy (CRT) on the frequency and functional activity of Th17 and Tc17 cells, as well as, on IL-17 mRNA expression in patients with CHF.

Methods Twenty-eight patients with CHF, analyzed before CRT (T0) and 6 months later (T6), and 15 healthy controls (HC) were enrolled in this study. Circulating Th17 and Tc17 cells were evaluated by flow cytometry. The quantification of IL-17A mRNA expression was performed by real-time PCR.

Results Circulating Tc17 cells tended to be higher in CHF patients submitted to CRT than in HC (0.92% (0.24–3.32) versus 0.60% (0.09–3.68), although not reaching statistical significance. The frequency of Tc17 cells in CHF patients significantly decreases after CRT reaching levels similar to those of HC (0.92% (0.24–3.32) at T0 versus 0.56% (0.21–4.20) at T6, $P < 0.05$), mainly due to responders to CRT. Additionally, the expression of IL-17 mRNA was detected in a few number of responder patients at T0 (27%) and only detected in one responder at T6 (7%). Conversely, in non-responders, the proportion of patients exhibiting IL-17 mRNA expression increases from baseline (17%) to T6 (42%). No significant differences were observed in Th17 cells between HC, CHF patients in T0 and patients in T6.

Conclusion The inflammatory response mediated by circulating IL-17 producing cells seems to be suppressed by CRT, particularly in responders.

Keywords Chronic heart failure · Cardiac resynchronization therapy · Th17 cell · Tc17 cell · Cytokines

1 Introduction

Heart failure (HF) is a complex clinic pathophysiological syndrome with a large impact on modern societies due to its high mortality and morbidity [1, 2]. The histological features of HF include loss of myocardial cells and restructuring of the extracellular matrix. Myocardial fibrosis may be caused by humoral factors as cytokines, growth factors, and hormones, suggesting that immunologic and inflammatory responses play a significant role in the development and progression of HF

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[3–7]. Pro-inflammatory cytokines may have an adverse impact on left ventricular function, due to a negative inotropic effect and induction of ventricular remodeling [8].

Peripheral blood mononuclear cells (PBMC), like T cells and monocytes, have been suggested as a potential source for extended systemic cytokine production in CHF [7, 9]. Like T helper (Th) 1 cells, Th17 cells were recently implicated in the pathogenesis of chronic inflammatory and autoimmune disorders, including rheumatoid arthritis, multiple sclerosis, psoriasis, and inflammatory bowel disease [10]. Furthermore, Th17 cells seem to possess more potent abilities to induce inflammatory diseases, comparing with Th1 cells, being described that interleukin (IL)17 plays an important role in coordinating tissue inflammation and up-regulating a range of pro-inflammatory mediators, including tumor necrosis factor (TNF)-α, IL-1, IL-6, IL-8, and matrix metalloproteinases [10, 11].

The pathogenic role of the Th1/Th2 imbalance in the development of CHF is well accepted, but the role of Th17 and of other IL-17 producing cells, remains unclear and contradictory data have been reported [12–14]. A Th17/Treg imbalance, characterized by an increased frequency of Th17 cells and decreased frequency of Treg cells, was described in CHF, suggesting a potential role of these cells in the pathogenesis of the disease [13]. On the contrary, other study showed no differences in circulating Th17 cells among CHF patients and healthy individuals [15].

Similar to Th17 cells, a new subpopulation of CD8+ T cells producing IL-17 (Tc17) cells were demonstrated to be increased in several chronic diseases, such as spontaneous urticaria, rheumatoid arthritis, and systemic lupus erythematosus [16–18]. In normal human peripheral blood, Tc17 cells are co-regulated with Th17 cells during differentiation [19, 20] and it seems to exist a cooperative or synergistic function between Th17 and Tc17 cells in T cell-mediated immunity [19]. These observations support the hypothesis of the participation of Tc17 cells in the inflammatory environment of CHF; however, the role Tc17 cells in CHF is still unknown.

Cardiac resynchronization therapy (CRT) is a well-recognized, highly effective device-based treatment for advanced CHF patients aiming to restore mechanical synchrony [21, 22]. However, at least 30% of patients receiving CRT do not respond to this therapy [23–25]. Moreover, some studies have shown a reduction in inflammatory mediators in HF patients treated with CRT [26–28] and, in small and medium sized trials, these observations were mainly observed in responder patients [29–31]. However, the impact of CRT in Th17 and Tc17 cells in CHF patients is still unknown. Therefore, we aimed to study the impact of CRT on IL-17-producing T cells, by evaluating the frequency and functional activity of Th17 and Tc17 cells in CHF patients submitted to CRT, as well as, IL-17 mRNA expression in circulating leukocytes.

2 Methods

2.1 Patient population

Twenty-eight patients with advanced heart failure scheduled for CRT, between 2010 and 2013, were prospectively enrolled in this study; their mean age was 60.3 ± 10.5 years, 18 patients were male and 10 were female (Table 1). Patients were assisted and followed up in the tertiary Cardiology Department, Centro Hospitalar e Universitário de Coimbra.

All patients were under stable, optimal pharmacological therapy for CHF at the time of inclusion, which includes an angiotensin-converting enzyme inhibitor or angiotensin receptor blocker, β-blocker, and aldosterone antagonist, unless contra-indicated or not tolerated. The inclusion criteria were as follows: class III or IV according to NYHA (New York Heart Association); left ventricular (LV) dysfunction with a LV ejection fraction (LVEF) ≤ 35%; QRS ≥ 120 ms with left bundle branch block; and normal sinus rhythm [24, 25, 32, 33]. The exclusion criteria included conditions that might influence the inflammatory response: clinical or biochemical manifestation of the presence of concomitant inflammatory disease; patients taking regular nonsteroidal anti-inflammatory drugs or patients on anticoagulants; active infections; known autoimmune or malignant diseases; severe valvular disease or congenital heart disease; cardiogenic shock; continuously or intermittently intravenous inotropic therapy; pregnancy; deep vein thrombosis or pulmonary embolism; severe peripheral arterial occlusive disease; severe and non-controlled arterial hypertension (systolic blood pressure > 180 mmHg or diastolic > 110 mmHg); comorbidities associated with a life expectancy less than 1 year; recent trauma or surgery (< 1 month); recent major bleeding (< 6 months) requiring blood transfusion; renal insufficiency (creatinine > 2.0 mg/dl); anemia (hemoglobin < 8.5 g/dl) or thrombocytopenia (< 100,000/L); atrial fibrillation; prior arterial coronary bypass surgery; acute coronary syndrome, or percutaneous coronary intervention within 3 months; previously implanted CRT system; and excessive alcohol consumption or illicit drug abuse.

A baseline assessment of heart failure patients (HFP) scheduled for CRT (T0) performed before the device implantation ensured candidate eligibility.

Six months after CRT (T6), the patients were re-evaluated for the same variables.

2.1.1 Echocardiographic evaluation

Each patient underwent echocardiographic assessment at T0 and T6. Standard echocardiography was performed using a Vivid 7 (GE Healthcare, Oslo, Norway) and 1.7-/3.4-MHz tissue harmonic transducer. Loops and three cardiac cycles were stored digitally and analyzed offline using a customized

Table 1 Characteristics of the heart failure patients enrolled in the study

	Global population, mean ± standard deviation, (*n* = 28)	Responders, mean ± standard deviation, (*n* = 15)	Non-responders, mean ± standard deviation, (*n* = 13)	*P* value
Baseline assessment				
Age (years)	61.3 ± 10.5	65.2 ± 9.6	56.8 ± 9.8	0.011
Gender (male/female)	18 / 10	9 / 6	9 / 4	0.989
Etiology (non-ischemic/-ischemic)	22 / 6	12 / 3	10/ 3	0.308
NYHA (III/IV)	24 / 4	14 / 1	10 /3	0.426
LVEF (%)	25.6 ± 7.0	24.9 ± 6.4	25.5 ± 7.6	0.274
LVESV (mL)	183.6 ± 95.8	178.4 ± 62.1	215.3 ± 124.6	0.465
LVEDV (mL)	235.5 ± 94.0	230.5 ± 64.0	264.1 ± 121.7	0.419
QRS	144.5 ± 31.0	138.8 ± 14.6	148.3 ± 38.6	0.447
hsCRP (mg/L)	5.8 ± 6.2	4.7 ± 4.3	7.0 ± 8.8	0.408
BNP (pg/mL)	262.9 ± 188.2	207.3 ± 126.1	324.2 ± 230.5	0.160
Cholesterol (mg/dL)	184.6 ± 58.9	191.1 ± 60.0	171.1 ± 45.3	0.338
Triglycerides (mg/dL)	134.5 ± 55.8	118.7 ± 51.0	143.1 ± 61.6	0.230
Uric acid (mg/dL)	6.2 ± 1.7	5.7 ± 1.6	6.7 ± 1.9	0.149
After CRT				
hsCRP (mg/L)	4.0 ± 4.6	2.7 ± 1.8	5.9 ± 6.7	0.118
BNP (pg/mL)	189.9 ± 295.0	80.3 ± 118.3	336.0 ± 395.3	0.033

NYHA, New York Heart Association; *LVEF*, left ventricular ejection fraction; *LVESV*, left ventricular end-systolic volume; *LVEDV*, left ventricular end-diastolic volume; *hsCRP*, high sensitivity C-reactive protein; *BNP*, B-type natriuretic peptide

software package (EchoPAC, GE Healthcare). The LV end-diastolic volume (LVEDV), LV end-systolic volume (LVESV), and LVEF were assessed by the biplane Simpson's equation in apical four-chamber and two-chamber views.

2.1.2 Definition of response to CRT

We classified responders to CRT as patients who were still alive and showed at least a 15% reduction in LVESV at the 6-months follow-up compared to baseline [34–37].

2.2 The healthy control group

The control group was composed by 15 sex- and age-matched healthy individuals (age 54 ± 12 years old; gender 7 females and 8 males). The inclusion criteria involved are the following: normal lipid profile, normal body mass, and normal cardiac evaluation. Exclusion criteria included the following: family history of heart disease and/or cardiomyopathy, active infections or inflammatory process, consumption of any drugs and/or alcohol, and inability to understand the informed consent.

2.3 Blood samples

Fasting blood samples were taken for chemistry assessment (including fasting glycaemia, creatinine, brain natriuretic peptide (BNP), high sensitivity C-reactive protein (hsCRP), and hematological parameters) in all patients, at admission, just before the device implantation. Peripheral blood (PB) samples were collected from each individual in heparinized and Paxgene tubes, at T0 and T6, to analyze the inflammatory parameters and IL-17 mRNA expression.

2.3.1 Multiparameter flow-cytometry immunophenotypic studies of Th17 and Tc17 subsets

In vitro stimulation of PB T cells was performed as described by others [18].

Briefly, 500 μL of each PB sample were diluted 1:1 (vol/vol), in RPMI-1640 medium (Gibco, Life Technologies, Paisley, Scotland, UK), supplemented with 2 mM L-glutamine. T cells were stimulated with 50 ng/mL of phorbol 12-myristate 13-acetate (PMA) (Sigma, Saint Louis, MO, USA) and 1 μg/mL of ionomycin (Sigma); after the addition of 10 μg/mL of brefeldin A (Sigma). The samples were incubated for 4 h at 37 °C, in a humidified incubator with 5% CO_2 concentration.

Each cultured PB sample was aliquoted in three different tubes (200 mL/tube) and incubated with the following monoclonal antibodies: anti-CD3 peridinin-chlorophyll proteins-cyanine 5.5 (PerCP-Cy5.5) (clone SK7; Becton Dickinson Biosciences (BD), San Jose, CA, USA) and anti-CD8 allophycocyanin (APC) (clone B9.11; Beckman Coulter—Immunotech, Marseille, France). Then, a cell permeabilization protocol, with IntraPrep Permeabilization Reagent (Beckman Coulter, Brea, CA, USA), and an intracytoplasmic staining protocol was followed, according to manufacturer's instructions, in order to analyze the intracellular expression of IL-17 conjugated with phycoerythrin (PE) (clone 41802; R&D Systems, McKinley Place, MN, USA). Cell aliquots were also stained separately with IL-2 (clone MQ1-17H12; BD Pharmingen, San Diego, CA, USA), TNF-α (clone MAb11; BD Pharmingen), and interferon (IFN)-γ (clone 4S.B3; BD Pharmingen), all conjugated with fluorescein isothiocyanate (FITC). Finally, cells were resuspended in 0.5 mL of phosphate buffer saline (PBS) (Gibco BRL, Life Technologies, Vienna, Austria) and then acquired in a flow cytometer.

2.3.2 Flow cytometry data acquisition and analysis

Data acquisition was performed in a FACSCalibur flow cytometer (BD) equipped with an argon ion laser and a red diode laser.

Among positive CD3 cells, CD4 positive T cells were identified by the absence of CD8; and CD8 positive T cells were identified by the co-expression of CD3 and CD8. The cytokine production (IL-2, TNF-α, and IFN-γ) was evaluated in IL-17 positive cells, within $CD4^+$ and $CD8^+$ T cells, on an electronic $CD3^+$ gate with at least 20,000 events, after a first acquisition step of 20,000 of total events.

Results illustrate the percentage of positive cells within each cell subset or/and their mean fluorescence intensity (MFI).

Data were analyzed using the Infinicyt™ software, V.1.5 (Cytognos SL, Salamanca, Spain).

2.3.3 Gene expression analysis

Analysis of IL-17A mRNA expression from whole blood was performed in blood collected in a PAXgene Blood RNA Tube (PreAnalytiX GmbH, Switzerland) with automated RNA purification in QIAcube (Qiagen, Hilden, Germany). One microgram of RNA was reverse transcribed with iScript TM Reverse Transcription Supermix for RTqPCR (Bio-Rad, Hercules, CA, USA), according to the manufacturer's instructions. Relative quantification of gene expression by real-time PCR was performed using a thermocycler (LightCycler 480 II; Roche, Basel, Switzerland). Normalization for gene expression quantification was performed with a geNorm Housekeeping Gene Selection Human Kit (Primer Design, Southampton, UK) and geNorm software (Center for Medical Genetics, Ghent University Hospital, Ghent, Belgium) to select optimal housekeeping genes for this study [38]. Real-time PCR reactions used specific QuantiTect Primer Assays (Qiagen) with optimized primers for IL-17A (QT00009233) and endogenous controls TOP1 (QT00068915) and SF3A1 (QT00061257), together with QuantiTect SYBR Green PCR Kit Gene expression (Qiagen), according to the manufacturer's instructions. Reactions were performed with the following thermal profile—10 min at 95 °C plus 50 cycles of 10 s at 95 °C, 20 s at 55 °C, and 30 s at 72 °C. Quantitative real-time PCR results were analyzed with LightCycler 480 software (Roche) and quantification was performed using the qBasePlus software package (Biogazelle, Zulte, Belgium).

2.4 Statistical analysis

Statistical analyses were performed using the non-parametric Mann–Whitney *U* test for independent variables. Wilcoxon signed-rank test was used to compare T0 vs. T6. Results were expressed as mean ± standard deviation or median (range). All statistical analyses were performed using Statistical Package for Social Sciences IBM SPSS 20 (IBM, Armonk, NY, USA) and GraphPad Prism version 5 (GraphPad Software, San Diego, CA, USA). Differences were considered to be statistically significant when the P value was < 0.05.

3 Results

3.1 Clinical evolution

The baseline characteristics of the studied population are described in Table 1. Before CRT, 14.3% of the patients ($n = 4$) were in NYHA class IV and 85.7% ($n = 24$) in class III. At the 6-month follow-up, the proportion of responders to CRT, according to the echocardiographic definition, was 55.6%.

As shown in Table 1, there were no statistically significant differences between responders and non-responders to CRT, regarding baseline characteristics. Despite the baseline longer QRS duration and the higher high sensitivity C-reactive protein (hs-CRP) and B-type natriuretic peptide (BNP) levels of non-responders by comparison to responders to CRT, these differences were not statistically significant at baseline assessment. After CRT, responders showed significant lower BNP levels compared with non-responders. During the 6-month follow-up, one HFP died (due to HF) and none has been transplanted.

3.2 Frequency of circulating Th17 and Tc17 cells in heart failure patients

As shown in Table 2, when considering all HF patients together, the frequency of Tc17 cells at baseline (HFP-

T0) displayed a tendency to be increased in comparison to the healthy group (HG). The percentage of Tc17 cells was also significantly higher at HFP-T0 compared to 6 months after CRT. Notably, at T6, the frequency of Tc17 decreased to the same levels observed in HG. In the same line, a slight increase of the amount of IL-17 produced at single-cell level (MFI) in those cells was observed in HF patients, both at T0 and T6, compared to the control group. No significant differences were observed in Th17 cells among the three studied groups. The decrease in the frequency of Tc17 cells from baseline to T6 is even more significant in responders to CRT. Regarding the comparison between responders and non-responders to CRT, we found no statistically significant differences in the frequency of Tc17 or Th17 cells, neither for the baseline values nor for the 6-month follow-up frequencies.

3.3 Functional characterization of peripheral blood Th17 and Tc17 cells from heart failure patients

Considering all HF patients together, no differences were found in the frequency of Th17 and Tc17 cells producing IL-2, TNF-α, or IFN-γ, neither when comparing HG and HFP, nor when comparing T0 and T6. However, responders to CRT presented at baseline an increased percentage of Tc cells co-expressing IFN-γ^+ and IL-17^+ compared to non-responders ($P = 0.045$) (Fig. 1).

3.4 IL-17 mRNA expression in whole peripheral blood cells from heart failure patients

IL-17 mRNA was detected in a small proportion of patients (22%, 6 out 27) and almost undetectable in HG (7%, 1 out 15) (Fig. 2a).

In responders to CRT, IL-17 mRNA expression was detected in a few number of patients at baseline (27%, 4 out 15) and only detected in one patient 6 months after CRT (7%, 1 out 15) (Fig. 2a, b). Conversely, in non-responders, the proportion of patients exhibiting IL-17 mRNA expression increases from baseline (17%, 2 out 12) to T6 (42%, 5 out 12) (Fig. 2a, b). Furthermore, this increase was associated to a higher IL-17 mRNA expression at T6 compared with baseline ($P = 0.043$) (Fig. 2a).

4 Discussion

CHF is characterized by a chronic inflammatory status. T cells seem to be part of the inflammatory response during CHF, independently of the etiology of the disorder [9]. To the best

Table 2 Frequency of Th17 cells among $CD4^+$ T cells and of Tc17 among $CD8^+$ T cells, and amount of IL-17 per cell (MFI) in the different groups, after *in vitro* stimulation with PMA plus ionomycin

	% Th17	IL-17 MFI in Th17 cells	% Tc17	IL-17 MFI in Tc17 cells
HG (n = 15)	1.83 (0.28–3.70)	79.06 (44.72–364.33)	0.60 (0.09–3.68)	57.45 (16.18–167.56)
P values HG vs HFP-T0	0.339	0.126	0.268	0.007
HFP-T0 (n = 28)	1.66 (0.52–6.22)	111.53 (55.32–245.52)	0.92 (0.24–3.32)	87.20 (31.02–210.71)
P values HFP-T0 vs. HFP-T6	0.716	0.509	0.026	0.412
HFP-T6 (n = 28)	1.80 (0.10–6.49)	92.70 (38.99–476.49)	0.56 (0.21–4.20)	88.75 (24.26–416.66)
P values HG vs HFP-T6	0.665	0.593	0.959	0.050
Responders (n = 15)				
P values HG vs HFP-T0	0.709	0.178	0.384	0.004
HFP-T0	1.39 (0.52–6.15)	100.61 (55.32–229.20)	0.91 (0.24–3.32)	117.82 (31.02–210.71)
P values HFP-T0 vs. HFP-T6	0.865	0.532	0.020	0.334
HFP-T6	1.67 (0.26–4.26)	80.46 (51.11–236.35)	0.37 (0.21–2.10)	83.35 (24.26–194.03)
P values HG vs HFP-T6	0.836	0.852	0.604	0.120
Non-responders (n = 13)				
P values HG vs HFP-T0	0.189	0.205	0.300	0.102
HFP-T0	2.02 (0.63–6.22)	122.45 (58.93–245.52)	1.08 (0.28–2.18)	71.80 (32.23–179.08)
P values HFP-T0 vs. HFP-T6	0.727	0.650	0.382	0.807
HFP-T6	1.92 (0.10–6.49)	117.80 (38.99–476.49)	0.64 (0.30–4.20)	88.94 (43.35–416.66)
P values HG vs HFP-T6	0.580	0.447	0.628	0.069

Results expressed as median (minimum-maximum). Statistically significant differences were considered when $P < 0.05$. Mann–Whitney *U* test was used to compare HFP-T0 versus HG and HFP-T6 versus HG. Wilcoxon signed-rank test was used to compare HFP-T0 versus HFP-T6. HG: Healthy control group; HFP-T0: Heart failure patients at baseline assessment; HFP-T6: Heart failure patients 6 months after cardiac resynchronization therapy (CRT) implantation

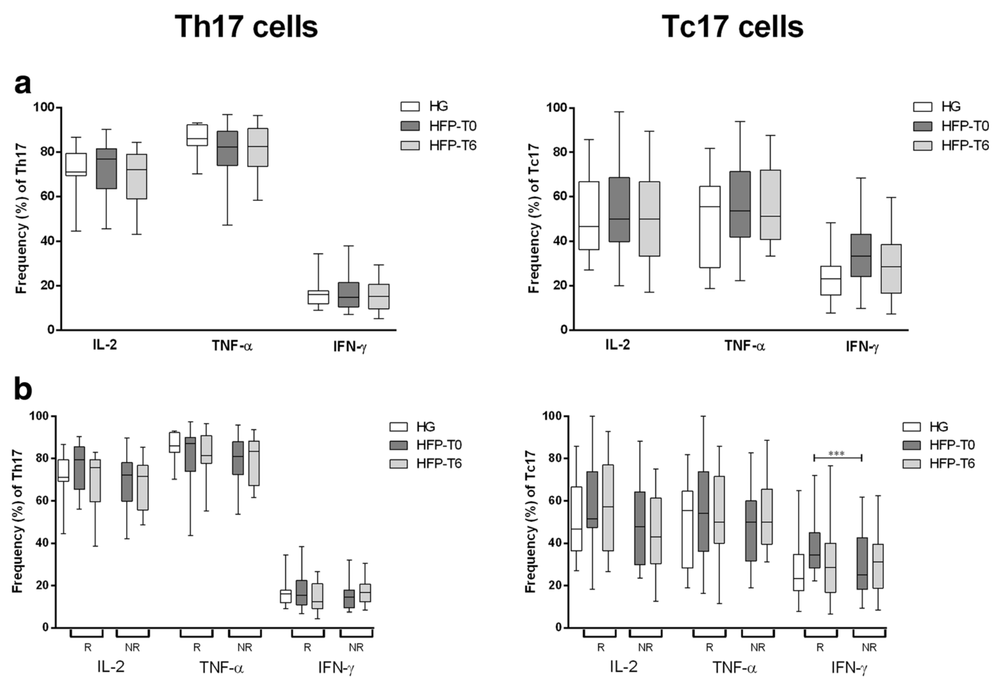

Fig. 1 Functional characterization of peripheral blood Th17 and Tc17 cells. The percentage of IL-2, TNF-α, and IFN-γ-producing Th17 and Tc17 cells was evaluated in healthy individuals (HG) and heart failure patients (HFP) distributed as follows: **a** total patients at baseline assessment (HFP-T0) and 6 months after cardiac resynchronization therapy implantation (HFP-T6); and **b** according to the response to cardiac resynchronization therapy: responders (R) and non-responders (NR) patients. ***Statistically significant differences were considered when $P < 0.05$

of our knowledge, this study is the first that has evaluated the impact of CRT on peripheral blood Th17 and Tc17 cells. Here, we demonstrate that responders to CRT exhibit a reduction in Tc17 cells reaching similar levels of healthy controls.

T cells are involved in the pathogenesis of the cardiac disease, both by direct cytotoxicity and by enhancing the inflammatory functions of other cells [39]. Th17 cells and/or IL-17 seem to be involved in the maintenance of chronic inflammation in CHF [13, 40–44], although there are no studies describing the role of Tc17 cells in heart diseases. Based in recent reports made by our group, describing the involvement of Tc17 cells in other inflammatory diseases, we considered relevant to study the behavior of both Th17 and Tc17 cells in patients with advanced HF submitted to CRT [16–18].

Multiple lines of evidence suggest crucial roles of IL-17 in cardiac pathology [40, 42, 43]. IL-17 can stimulate epithelial cells, endothelial cells, fibroblasts, and other cells to release massive cytokines such as granulocyte colony-stimulating factor (G-CSF), IL-6, and matrix metalloproteinase [40, 41]. Consequently, IL-17 can inhibit the reconstruction of the heart by myocardial fibrosis through dissolution, breakage, and reduced synthesis of intercellular collagen. Studies made in animal models raised the hypothesis that IL-17 can cause damage in the heart through several mechanisms: through direct toxic effects on myocardial cells, reduction in myocardial intracellular calcium levels, and enhancement of the activity of pro-inflammatory cytokines such IL-6 and IL-1β. These mechanisms may lead to cardiac hypertrophy, increased cellular necrosis, accelerated myocardial apoptosis, and extracellular matrix remodeling and, thus, accelerate heart failure progression [41].

CRT can reduce both the morbidity and mortality in a subset of patients with HF; however, it is not well known how CRT affects the immune system. One study made in a small cohort of patients with HF reports a decrease of inflammatory markers, as IL-8, IL-6, monocyte chemoattractant protein 1 (MCP-1), and B-type natriuretic peptide (BNP), 6 months after CRT [45]. In our work, levels of Th17 cells in HFP were similar to the control group levels. This finding is in agreement with another report that describes comparable results in circulating Th17 cells frequencies, serum IL-17 levels, and ROR-γt expression between CHF patients and healthy individuals [15]. Considering Tc17 cells, we observed a trend to an augmented frequency among HFP, compared to healthy controls, accompanied by an increased amount of IL-17 produced per cell (MFI) in HFP. When we evaluated the same HF patients 6 months after CRT, we found a significant decrease in Tc17 frequency. Notably, this decrease is mainly due to the responder group, which showed a significant decrease in the frequency of Tc17 cells from baseline (T0) to T6.

These achievements seemed to be in agreement with the lower number of responder patients in whom had been detected IL-17 mRNA expression and the maintenance/decrease of mRNA levels observed. On contrary, we found a higher number of cases with detectable IL-17 mRNA, as well as, an increase of IL-17 mRNA expression between T6 and T0, in non-responders patients.

The decrease of IL-17-producing T cells in HFP after CRT implantation is possibly related to the reduction of the inflammatory process inherent to CHF and, probably, with the improvement of the cardiac function. The definition of response to CRT based on LV reverse remodeling has been used in the

a

	% of cases expressing IL-17 mRNA (n)	Median (minimum-maximum)*
HG	7% (1/15)	1.74 (-)
HFP-T0	22% (6/27)	0.65 (0.28-0.75)
HFP-T6	22% (6/27)	1.99 (0.32-5.35)
According to response to cardiac resynchronization therapy		
HFP-T0	27% (4/15)	0.65 (0.28-0.75)
HFP-T6	7% (1/15)	0.77 (-)
HFP-T0	17% (2/12)	0.61 (0.51-0.71)***
HFP-T6	42% (5/12)	2.18 (0.32-5.35)

b

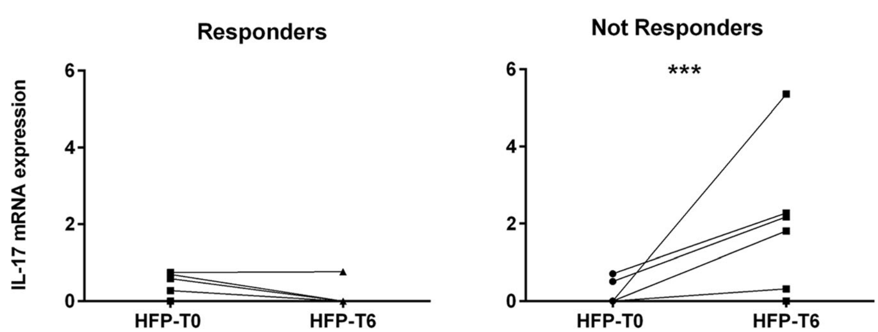

Fig. 2 **a** IL-17 mRNA expression in whole peripheral blood cells from healthy individuals (HG, *n* = 15) and heart failure patients (*n* = 27), distributed according to the response to cardiac resynchronization therapy. The results are expressed as percentage (number of cases) wherein mRNA expression was detected by RTPCR. *Only considering the samples in which IL-17 mRNA was detected. **b** IL-17 mRNA expression on whole PB cells from heart failure patients distributed according to the response to cardiac resynchronization therapy. ***Statistical significant differences were considered when *P* < 0.05

major clinical trials on CRT. However, the final objective of CRT is to prolong survival and/or to alleviate heart failure symptoms and a positive reverse remodeling response to CRT does not necessarily parallel a favorable outcome or symptomatic benefit [46]. Therefore, we may speculate that the anti-inflammatory effect of CRT could be a useful complementary marker to include in a composite definition of response to CRT.

From a functional point of view, Th17 cells are largely defined by the production of IL-17A and IL-17F, which have the ability to recruit neutrophil, possessing a pro-inflammatory function [47]. Th17 cells can also produce several other inflammatory cytokines such as IL-21, IL-22, IL-6, TNF-α, and IFN-γ [48]. In the failing heart, elevated left ventricular end-diastolic wall stress causes myocardial expression of cytokines, which directly or indirectly influence left ventricular contractile performance and remodeling. The pro-inflammatory cytokines, namely TNF-α, IL-1, and IL-6, lead to monocyte activation, while IL-2 leads to T cells activation. Taken together, they promote monocyte-endothelial cell adhesive interaction, with subsequent cytokine production and free radical generation, giving rise to inflammation, tissue destruction, cardiovascular remodeling, and loss of function [49]. In an attempt to assess the impact of CRT on the functional inflammatory responses of Th17 and Tc17 cells in patients with CHF, we analyzed the frequency of these cells producing not only IL-17 but also IL-2, TNF-α, and IFN-γ, after stimulation *in vitro*. We found no significant differences in the expression of IL-2, TNF-α, and IFN-γ by Th17 and Tc17 cells between healthy individuals and HFP, in both moments of evaluation, but responder patients presented a significantly higher expression of IFN-γ by Tc17 cells. Tc17 cells possess a high plasticity and can convert to IL-17/IFN-γ-double producing cells (Tc17/IFN-γ cells), permitted by IL-12 signaling, with distinct properties from Tc1 lineage. In addition to their highly cytotoxic and antitumor activity, Tc17/IFN-γ cells were found to be implicated in various inflammatory conditions in human and animal models [16, 17, 50].

In the case of T cell-mediated inflammation in the heart, IFN-γ has a complex combination of pro-inflammatory and anti-inflammatory effects, including a feedback inhibition of T cell activation and effector functions [39]. Since Tc 17 cells display a greater plasticity of the cytokine producing phenotype than their Th17 counterparts [51], we can speculate that this could translate into a different pathogenic role of Tc17 cells in CHF and could explain why only Tc17 cells are reduced by CRT.

Taken together, our study raises novel insights on the impact of CRT over the immune behavior of Th17 and Tc17 cells in advanced HF. The inflammatory response mediated by IL-17 producing cells seems to be effectively reduced by CRT, particularly Tc17, and this immune benefit may contribute to the positive response to CRT.

4.1 Limitations

The main limitation of our study is the small sample size. However, since the evaluation was performed in two different moments (before and after CRT), this study has the strength that each patient served as his own control. Another limitation is the short follow-up. As inflammation was not re-evaluated after the 6-month follow-up, we do not know whether the possible anti-inflammatory effect of CRT is sustained over time. Finally, we did not investigate whether changes in IL-17-producing cells were associated with clinical benefit after CRT and further studies are required to evaluate if the alleviation of inflammatory status translates into improved prognosis after CRT.

Funding information This study received financial support from the PEst-OE/SAU/UI0709/2014 project.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The study was performed in accordance with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards and the research protocol was approved by the local Ethical Committee.

Informed consent All the studied patients gave and signed the informed consent.

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